vector. The vector used in the CGD study had much higher transcriptional activity in HSPCs than the vector used in most other trials[7]. Although this strong activity probably allowed better reconstitution of the NADPH enzyme activity, it also may have provided a greater capacity to transactivate cellular genes at the integration site, and thus a higher chance of oncogene activation. The vector could also possibly confer some specificity to the target gene and host cell undergoing activation.

The source of transduced cells also may make a difference. The CGD study used growth factor–mobilized peripheral blood HSPCs, which are more enriched in activated myeloid progenitors than the bone marrow cells used in other trials. Thus, a different array of progenitor subsets, and possibly a specific activation state, may have influenced the gene transfer frequency and the integration site selection to favor cells more susceptible to *EVI1*-induced immortalization.

The findings of Grez, von Kalle and colleagues strengthen the emerging concept that the type and frequency of side effects of retroviral insertional mutagenesis depend highly on context. The study also highlights the need to pay more attention to all the variables in gene transfer protocols besides the disease type and transgene function. Rather than dismissing gene therapy for falling short of its early enthusiastic promises, we should start to recognize the medical advances and the scientific insights born out of its painful coming of age.

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Fighting infections with vitamin D

Michael Zasloff

Sunlight can treat tuberculosis, a phenomenon observed more than a century ago. The mechanism now becomes more clear, and it involves induction of a microbe-fighting peptide by vitamin D.

In 1895, Niels Finsen of Denmark found an effective way to treat tuberculosis. He exposed individuals with tuberculosis of the skin—lupus vulgaris—to high-intensity light produced from an electric arc lamp. Exposing a small area of affected skin to intense light produced moderate sunburn. The superficial skin layers subsequently peeled away—leaving normal, healthy skin underneath. Phototherapy cured or substantially improved the disease in about 95% of affected people, and by the 1920s sun exposure for the treatment of pulmonary tuberculosis had become routine.

Finsen was awarded a Nobel Prize in 1903 for his treatment, preceding Robert Koch who claimed the prize in 1905 for identifying the causative agent of tuberculosis, and Selman Waksman, who won in 1952 for his discovery of streptomycin, the first antibiotic to cure the disease.

More than a century after Finsen's discovery, we are beginning to understand how sunlight helps us battle tuberculosis and other microbes. In a recent issue of *Science*, Liu *et al.*[1] propose that sunlight, by stimulating the synthesis of vitamin D, upregulates the expression of a microbe-fighting peptide.

Most multicellular organisms produce antimicrobial peptides (AMPs) and proteins, which can kill viruses, fungi, protozoa, bacteria and other microbes. In people, AMPs are produced on epithelial surfaces and within circulating white cells[2,3]. Some AMPs are expressed constitutively, and others are expressed in response to stimuli such as tissue injury (through interleukin-1 and other cytokines) or microbial components (such as lipopolysaccharide). Amongst the better studied of the inducible AMPs are human β-defensins 2 and 3 and LL-37 (also known as cathelicidin)[4].

In addition to their anti-infective activities, AMPs such as LL-37 help orchestrate the ensuing wound-repair process. LL-37 stimulates local angiogenesis and synergizes with the epidermal growth factor receptor to promote epithelial growth. LL-37 can also attract monocytes and neutrophils through FMLP receptors on these cells[2,3].

Recent studies of the gene encoding LL-37 have revealed that it contains sites for the vitamin D receptor (VDR)[5–7]. The active form of vitamin D—1,25-D3—boosts levels of LL-37 in human neutrophils. 1,25-D3 also induces expression of LL-37 in keratinocytes in tissue culture and after topical administration onto the skin of human subjects[8].

Why should an antimicrobial peptide be induced by vitamin D, a product of sunlight?

Sunlight, especially within the UVB spectrum, 'burns' skin, by damaging the lipids and DNA of epidermal cells[9]. At the same time, sunlight also induces the synthesis of two powerful immunosuppressants within the skin, vitamin D and α-MSH. α-MSH is believed to exert its anti-inflammatory effects by inhibiting the activation of NF-κB in a wide spectrum of tissues. Vitamin D depresses the activity of Langerhans cells, and inhibits the induction of T helper type 1 cells and the expression of major histocompatibility complex (MHC) class II proteins on antigen-presenting cells.

We assume that UVB-induced immunosuppression evolved to control the intensity of inflammation (for example, pain, redness, epidermal damage) caused by UVB-provoked injury. But suppressing inflammation also makes us more vulnerable to infection, especially in a setting where the skin has been damaged. To balance this compromise in host defense, vitamin D stimulates the synthesis of the potent antimicrobial peptide LL-37 in skin and circulating phagocytic cells.

Liu *et al.* did not set out to explain the antimicrobial properties of sunlight. The authors were exploring the response of human immune cells to activation by the Toll-like receptor (TLR) 2/1, which senses molecules derived from pathogens. They observed that activated monocytes or macrophages could

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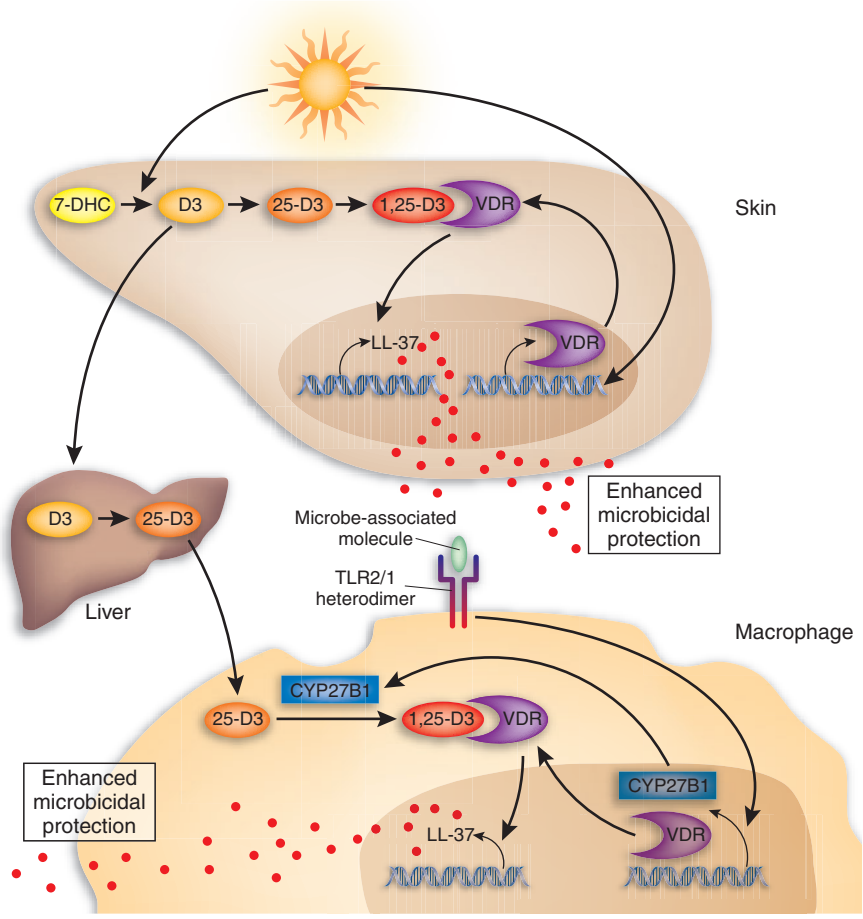

Katie Ris

Figure 1 Fighting infections with vitamin D. Two related pathways within the skin and circulating monocytes-macrophages are highlighted. Sunlight converts 7-dehydrocholesterol (7-DHC) in the skin to vitamin D3, which is converted successively to 25-hydroxy-D3 (25-D3) and then to 1,25-dihydroxy-D3 (1,25-D3) within keratinocytes. Sunlight also induces expression of the vitamin D receptor (VDR). 1,25-D3 and the VDR then together induce the expression of the gene encoding the human antimicrobial peptide LL-37. Vitamin D3 enters the systemic circulation and is converted to 25-D3 by the liver. Circulating monocytes are activated by TLR2/1 agonists present on specific microbes. The genes encoding VDR and CYP 27B1 are induced. CYP27B1 converts 25-D3 from the circulation to 1,25-D3, joins with the VDR and activates the gene encoding LL-37, leading to an increase in cellular LL-37 and enhanced microbicidal activity of the phagocyte.

effectively kill *Mycobacterium tuberculosis*, whereas activated dendritic cells could not. They set out to determine the basis of this difference, and compared the gene expression profiles of the two cell types. They discovered that two genes were uniquely expressed in activated monocytes: the VDR and CYP 27B1, the enzyme that converts 25-D3 into 1,25-D3, the form of vitamin D that binds to the VDR.

Liu *et al.* then used bioinformatic tools to help identify human genes that were both activated by vitamin D and known to express products with antimicrobial properties. LL-37 was a prime candidate. Indeed, activated monocytes incubated with 1,25-D3 produced LL-37, whereas similarly treated dendritic cells did not.

LL-37 concentrated within phagocytic vacuoles containing engulfed bacteria, and as expected, killed *M. tuberculosis in vitro*. Moreover, macrophages more effectively killed ingested *M. tuberculosis* after they were exposed to 1,25-D3.

Liu *et al.* conclude that stimulation of TLR2/1 on human monocytes engages a vitamin D–dependent intracellular circuit that results in the expression of LL-37. The net result is enhanced microbicidal capability of the monocyte. This study tells us that sunlight, by raising serum levels of vitamin D, can increase the capacity of circulating monocytes and macrophages to kill certain microbes they are exposed to (**Fig. 1**).

Liu *et al.* suggest that their observations might explain why American blacks appear to be particularly susceptible to infection by *M. tuberculosis*. Previous studies have shown that American blacks have substantially lower serum vitamin D levels than whites, as a result of the greater UV shielding afforded by their skin's higher melanin content. Liu *et al.* confirm this observation. In addition, they show that activated monocytes incubated with the serum of white subjects contained more than twice as much cathelicidin—the precursor of LL-37—than the same monocytes incubated with serum from black subjects. The authors next supplemented serum from blacks with 25-D3 to concentrations observed in the serum of whites. This supplemented serum boosted cathelicidin levels in the activated macrophages to levels observed in monocytes collected from whites. The authors suggest, but do not demonstrate, that activated monocytes functioning within blacks are likely to be less effective at killing *M. tuberculosis* than monocytes from whites. If so, then treatment with 25-D3 should augment the microbicidal capacity of monocytes from blacks.

The study also highlights the difference between mice and humans. Mice appear to rely heavily on nitric oxide, generated within activated macrophages and neutrophils, to kill microbes ingested by these cells. Curiously, the gene encoding mouse cathelicidin does not contain a VDR binding site, nor is the gene inducible by vitamin D[7]. Liu *et al.* suggest an evolutionary explanation: mice are nocturnal, whereas humans have adapted to living in sunlight.

The study of Liu *et al.* and other recent studies provoke questions about what constitutes 'appropriate' doses of sunlight and vitamin D. Should our minimal daily requirement of vitamin D be adjusted on the basis of skin pigmentation?[10].

We currently base vitamin D requirements on amounts required to sustain optimal health of our skeleton. The studies reported here suggest that optimal functioning of our innate immune system might require more vitamin D. Considering the broader immunomodulatory properties of vitamin D, we wonder whether low plasma levels of vitamin D might underlie the increased incidence of kidney transplant rejection in blacks compared to whites, attributed in part to unexplained "immunologic hyper-responsiveness"[11].

Certain drugs inhibit CYP 27B1, such as the antifungal ketoconazole and the HIV-protease inhibitor ritonavir[12]. Do these anti-infective agents compromise 1,25-D3 synthesis within monocytes and thereby depress their microbicidal capacity? Should we be examining the therapeutic benefits of vitamin D for acute bacterial or viral infections, as others have suggested[5–7]? Should we consider administering vitamin D to people with conditions associated with depressed expression of LL-37, such as *Shigella* dysentery, atopic dermatitis or burns[2,3]?

Perhaps in the future we might be able to treat or prevent certain infectious diseases with safe

and inexpensive substances that induce expression of endogenous antimicrobial peptides[2,3].

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Remodeling after stroke

Berislav V Zlokovic

A promising approach to treating ischemic stroke, inhibition of matrix metalloproteinases (MMPs), may need to be rethought. Previous work suggested that inhibitors of MMPs could protect the brain, but now it seems that such inhibitors might contribute to damage (pages 441–445).

Shortly after an ischemic stroke, matrix metalloproteinases (MMPs) seem to contribute to subsequent brain damage by proteolitically degrading neurovascular matrix, which may result in brain hemorrhage and neuronal apoptosis. In some experimental situations, inhibition of MMPs protects against such damage, which has created interest in MMP inhibitors as drugs for stroke.

Findings in this issue sound a note of caution for such efforts. Like many biological mediators, MMPs can be put to use for good or bad, and that also seems to be the case in the brain. Although MMPs seem to contribute to damage early after a stroke, Zhao *et al.*[1] find that MMPs take on another role as the days progress—they help mediate repair. The authors show that inhibition of MMPs, particularly MMP-9, during this later time window hinders brain repair in mice and may even increase the risk for intracerebral bleeding.

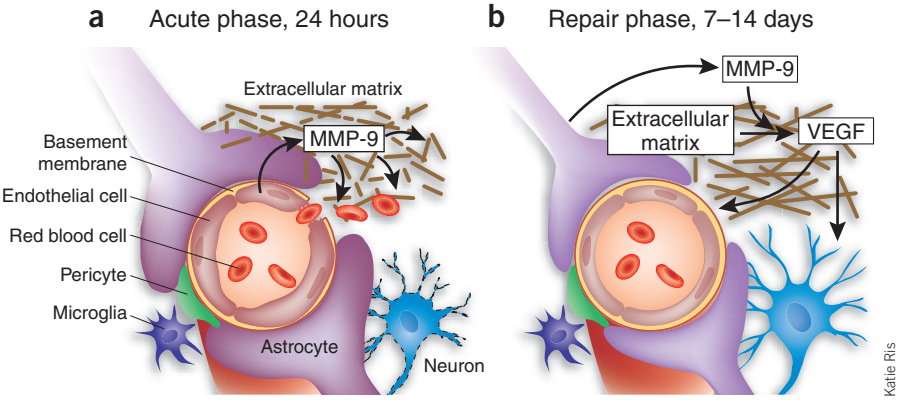

Figure 1 Redistribution of MMP-9 within the neurovascular unit after stroke. (**a**) Acute phase: disruption of the blood-brain barrier, neuronal apoptosis and hemorrhage are mediated by brain endothelial secretion of MMP-9, which degrades the neurovascular extracellular matrix and the basement membrane. (**b**) Repair phase: remodeling of the neurovascular unit requires MMP-9–mediated processing of vascular endothelial growth factor (VEGF) and release of biologically active VEGF from the extracellular matrix. MMP-9 is mainly secreted by astrocytes and neurons, whereas VEGF drives neurovascular regeneration.

MMPs, also known as metalloproteases or matrixins, are a family of secreted and membrane-bound proteases that are crucial for many biological processes including embryogenesis, normal tissue remodeling, wound healing and angiogenesis[2]. To date, more than 25 different vertebrate MMPs have been identified. Most are multidomain proteins with multiple biological functions including degradation of the proteins of the extracellular matrix (ECM) to facilitate cell migration during development and tissue regeneration; remodeling of newly synthesized ECM; regulation of the inflammatory response through proteolytic processing of cytokines and chemokines; and release and processing of ECM-bound growth factors, resulting in their activation[2–4]. To achieve optimal enzymatic activity, MMPs are tightly controlled at the transcriptional level, as well as at the protein level through activation by their zymogens and inhibition by tissue-specific inhibitors[2–5]. A loss of control over the activity of MMPs results in a variety of diseases such as arthritis, cancer, cardiovascular disorders, nephritis, tissue ulcers, fibrosis and neurodegeneration[2,3].

MMPs may have both beneficial and detrimental effects on developing brain and adult brain and spinal cord[4,5]. Detrimental effects include dysfunction of the blood-brain barrier (BBB), demyelination, neuroinflammation, neurotoxicity, and roles in development of pathology in spinal cord injury, multiple sclerosis and stroke. Beneficial effects include roles in the development and neurogenesis of the central nervous system, growth and regeneration of axons, myelogenesis, angiogenesis and termination of neuroinflammation.

Many MMP inhibitors have been synthesized over the past 15 years, with the focus directed at preventing cancer and rheumatoid arthritis[2,3]. Still, the major challenge with the therapeutic interventions of MMPs remains how to accomplish temporal and spatial control of their activity in a target organ, tissue or system. Blocking MMPs at a badly chosen time and in nontarget cell types may result in unwanted side effects.

In contrast to the findings of Zhao *et al.*[1], earlier studies from several laboratories supported the view that inhibiting MMP-9 after stroke is neuroprotective[4,6,7]. Inhibition of MMP-9 in mice, for instance, prevents proteolysis of neuronal laminin, which protects neurons from apoptosis—whereas lack of MMP-9 stabilizes the BBB by preventing degradation of BBB-associated proteins[8]. These

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