JON CARTWRIGHT

This fruit represents skin, with soft dermis underlying layers of keratinocytes at varying stages of maturity.

IMMUNOLOGY

A many layered thing

No mere passive barrier, the skin is being revealed to be an active part of the immune system. Researchers are now starting to understand its role in driving psoriasis.

BY CLAIRE AINSWORTH

While examining the skin of his psoriasis trial patients, Jim Krueger realized that some of his basic assumptions about the disease were wrong. The year was 1994, and Krueger, then a cell biologist at the Rockefeller University in New York, had been studying psoriasis in order to better understand skin cancer. The prevailing idea at the time was that excessive division of skin cells caused psoriasis, with chronic inflammation as a side effect. For Krueger, the disease was a logical model for studying skin malignancies, where cell division is key.

As part of the study[1], Krueger's team had given patients a drug that targeted certain immune cells but left skin cells unaffected. Much to his surprise, their skin markedly improved — in some cases, lesions all but vanished. This effect implicated immune-system cells as the chief instigators of the disease, rather than as secondary players. It also scotched Krueger's idea of using psoriasis as a model for diseases driven by abnormal skin cell division. "I suppose as a scientist if you disprove your hypothesis you've done the best job you can," Krueger observes. "I disproved my hypothesis — and that led me to work on the immunology of skin disease."

The finding was not only a turning point for Krueger's research, but also a major development for psoriasis and skin immunology more broadly. Although evidence had been mounting that the immune system was involved in psoriasis, Krueger's study was the first to discriminate unequivocally between the roles played by keratinocyte cells, which make up most of the skin's physical barrier, and T cells, which help direct the adaptive arm of the immune system. Adaptive immunity can specifically target and remember particular pathogens; in contrast, the innate immune system produces molecules that have a general, non-specific antimicrobial activity.

Over the past two decades, immunologists and psoriasis researchers have shown not only that both arms of the immune system are present in the skin, but that the skin itself is an immunologically active organ. Readily accessible and easy to observe, psoriasis is also shaping up as a model for other chronic inflammatory conditions, such as Crohn's disease and rheumatoid arthritis. Thanks to technological developments in molecular biology and genetics, researchers hope that detailed understanding of the skin's immunology may finally be within reach. "We are going to map chronic inflammation much better than we ever could before," says Frank Nestle, a clinician and immunologist at King's College London.

IMMUNE CELLS

The classical picture of the skin is of a layered barrier that shields the body from the insults of the surrounding world while holding on to moisture and heat. The outer layer, or epidermis, forms the main defence against the outside world; the underlying, cushioning layer, or dermis, contains blood and lymphatic vessels, fat cells and fibroblasts, hair follicles, sweat glands and sensory structures such as touch-sensitive mechanoreceptors (see 'Psoriasis uncovered', page S50).

The epidermis is formed of layers of keratinocytes at varying stages of maturity. Those in the deepest layer are dividing, supplying new keratinocytes that progressively differentiate and rise. By the time they reach the outermost layer they are dead, having become tough, flat, water-retaining husks called corneocytes.

By the 1980s researchers had found that immune-system cells beat a regular path

between the lymphatic system and the skin. As a result, the classical picture of the skin as a passive barrier started to morph into one of an immunologically active organ. Langerhans cells, which are specific to the epidermis but whose function had been unclear for many decades, were recognized as being part of the immune system. In fact, they are a type of dendritic cell that samples the environment and presents fragments of protein to other immune cells as part of the pathogen surveillance system.

The discoveries continued with more kinds of dendritic cell, this time in the dermis. The dermis is now understood to be host to an array of immune cells. Among them are different T-cell types from the adaptive immune system, such as $CD4^+$ helper cells that coordinate immune responses and $CD8^+$ killer cells that destroy infected, damaged or abnormal body cells. Also present are cells that form part of the innate immune system: natural killer cells, which destroy foreign cells; mast cells, which release inflammation-triggering molecules such as histamine; and macrophages, which gobble up microorganisms and cellular debris. Other dermal immune cells secrete a signalling network of cytokines that coordinate the immune response.

In this milieu, keratinocytes had been thought of simply as the cells that form and renew the skin's barrier. However, in the past few years, it has become clear that they also play a key role in the skin's immune response (see 'Cell summoners').

SYSTEM RESET

If the skin is infected or wounded, the inflammatory response prompts immune cells to produce growth factors that promote both keratinocyte cell division and wound closure. In normal skin, the inflammation resolves once the injury has healed or the infection has cleared. In psoriasis patients, once inflammation has been triggered, it persists. Recent research, plus a hint of serendipity, has helped paint a picture of the major factors involved (see 'Caught in a loop').

Krueger's surprise finding in 1994 revealed the role of T cells in psoriasis. Revelation of the involvement of the tumour necrosis factor (TNF)-α cytokine came from a lucky observation reported[2] in 2000. A patient receiving treatment for inflammatory bowel disease with a drug that blocks the activity of TNF-α saw her psoriasis dramatically improve. Since then, several TNF-α-blocking drugs, many of which have been co-opted from other autoimmune conditions, have proved successful in treating psoriasis, although they have harsh side effects. The generation of drugs now in clinical trials that target other cytokines (see 'Silencing psoriasis', page S58) is the direct result of this process of studying the immunobiology of psoriasis, says Nestle.

Although these next-generation drugs seem to be good at controlling psoriasis symptoms, none yet corrects the underlying mechanism: even if treated skin looks healthy, its gene activity can still be abnormal. "You only pull the plug on the inflammatory disease," says Nestle, "you don't reset the immune system."

Resetting the immune system will require a more comprehensive view of how the disease works and what triggers it. With this aim in mind, researchers have turned to '-omics' technologies — genomics, transcriptomics and proteomics — to reveal how biological activity in psoriatic skin compares with that of healthy skin, and how this activity changes after drug treatment.

The transcriptome consists of all the RNA that a cell produces, and is a measure of gene activity. Comparing the transcriptomes of skin cells from psoriatic lesions with those from non-psoriatic skin in the same patient shows differences in gene expression. Several such studies have been published, but they used different patient populations and methodologies, making it hard for researchers to define a core set of common genes simply by looking for overlaps in the genes identified. "There's clearly a complex inflammatory network going on in the skin," says Krueger.

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To address this problem, Krueger's team performed a meta-analysis[3] on five transcriptomics studies, applying a series of statistical models to the combined data. They identified more than 1,000 genes that were expressed at different levels in psoriatic skin and normal skin. The team hopes the genes will act as a reference list for future work, as well as providing leads for understanding the pathogenesis of psoriasis and developing new therapies and methods of monitoring response to therapy.

The next big challenge is to make sense of the vast amounts of information being generated. Researchers are turning to computer models to assemble a comprehensive picture of the intricate network of molecular interactions. This systems immunology approach should allow researchers to pinpoint hubs: those molecules that are vital for maintaining a network in a stable configuration. Altering the activity of a hub can abruptly shift the behaviour of a network from one state to another. New drugs that target hubs have the potential to switch a network from a disease-associated state to a healthy one. The result could be treatments that don't just treat the symptoms of psoriasis but actually correct the underlying disease process.

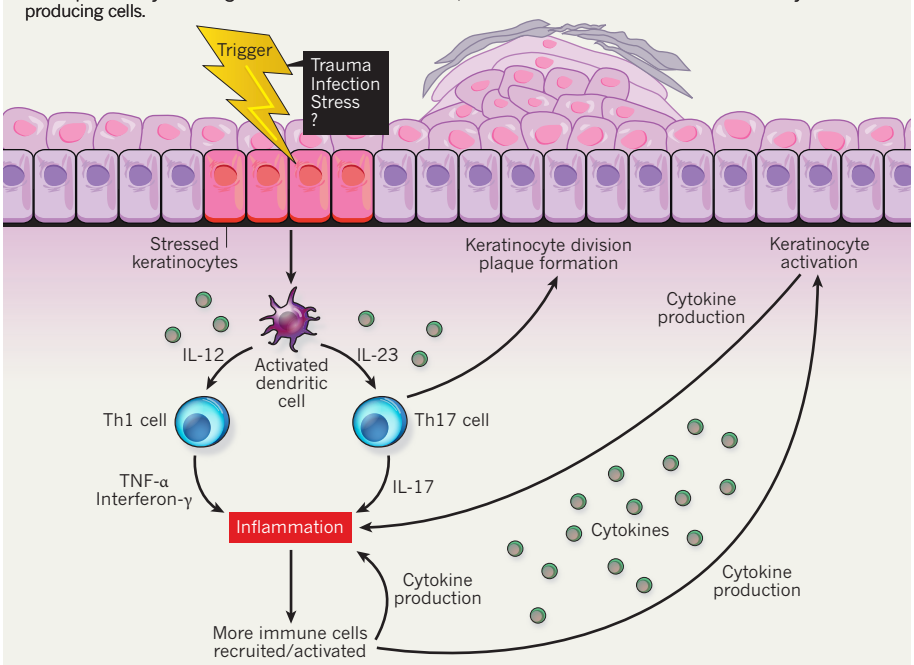
NETWORKING

Although classical genetics and cell biology have provided valuable insights into the interactions in cells and tissues that characterize psoriasis, such approaches are slow and reveal only localized relationships. To build a more complete picture of the whole network, researchers are adopting one of two approaches to sketch out potential molecular interactions and network topologies.

One tack is to identify factors that are regularly found together in the same tissue, indicating a potentially dependent relationship.

CAUGHT IN A LOOP

A number of triggers can cause keratinocytes to become stressed, resulting in the activation of immune cells. These produce cytokine signals that cause inflammation, which in turn recruits and activates more cytokine-producing cells.



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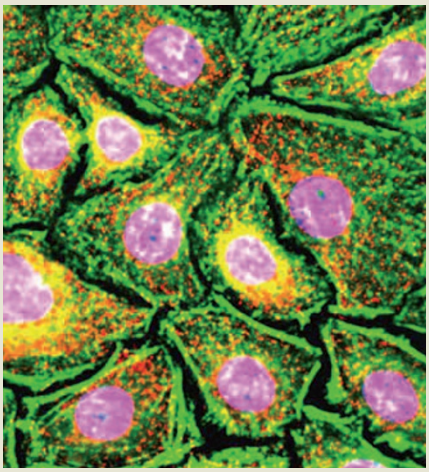
CELL SUMMONERS

Keratinoctyes are more than just barrier cells

Since the 1980s, researchers have known that keratinocytes (the cells that make up most of the epidermis) produce antimicrobial peptides — fragments of protein that target and kill disease-causing microorganisms. It is only over the past 5 years, however, that it has become apparent just how big a bag of immunological tricks the keratinocytes have at their disposal.

Keratinocytes express several receptors, including Toll-like receptors (TLRs), which sense the presence of molecules common to many microbes. TLRs play a major role in the innate immune response: the general, non-specific reaction to pathogens. Keratinocytes also release signalling molecules called cytokines that activate other immune cells.

This close confederacy between keratinocytes and the immune system not only defends the skin against infections, but also helps it heal when wounded. In normal skin, cytokines direct the development of keratinocytes to promote skin regrowth and wound closure. In psoriasis, abnormal cytokine secretion retards keratinocyte differentiation, leading to thickened, scaly plaques. "What's going on in psoriasis is that there is chronic expression of this alternate development pathway," says Jim Krueger, an immunologist and clinician at the Rockefeller University in New York. "It becomes a self-perpetuating loop."



Keratinocytes dyed to show f-actin (green), nuclei (pink) and proteins called defensins (yellow).

R. BICK, B. POINDEXTER, UT MEDICAL SCHOOL/SCIENCE PHOTO LIBRARY

However, it is not yet clear how involved keratinocytes are in perpetuating psoriasis. In about 10% of patients, says Krueger, small injuries such as scratches can trigger the formation of new psoriatic plaques, a phenomenon known as the Koebner response. This suggests that keratinocytes have the ability to initiate local patches of chronic inflammation by summoning and activating immune cells.

This ability could be related to the role of keratinocytes in wound healing. In 2012, Krueger's group reported[6] that they had mimicked wound healing by separating keratinocytes from other skin cells. By observing the resulting changes in gene expression, they found that keratinocytes not only make cytokines that attract innate immune cells, but they also summon adaptive immune cells, including T cells. "The more we understand about what keratinocytes make," says Krueger, "the more we can understand about the protective function that the skin has." **C.A.**

A computer program searches for genes, proteins and other molecules that are coexpressed and that might point to common biochemical pathways. Nestle has used this approach to mine gene-expression data sets to build a psoriasis 'interactome' (networks of genes that have similar expression patterns in different tissues and samples). The team hopes the interactome, which they are preparing to publish, will serve as a reference network for interpreting the results of other studies and help uncover new disease mechanisms.

However, this approach will only reveal which molecules are interacting; the next step is to explore the biological consequences of these interactions. This is where the second approach — mathematical modelling — comes in. Nestle's team, for example, has built a mathematical model[4] of the immune-cell interactions and cytokine networks in psoriasis. The model shows how changes in cytokine concentrations can alter the inflammatory response. People with a genetic susceptibility to psoriasis secrete more cytokines than those without; Nestle's model predicts that such a cytokine glut will form a stable network, but one that is prone to suddenly switching states. A trigger, such as a bacterial infection, could tip it into another stable state: chronic inflammation. "In individuals who don't have the susceptibility, the inflammation resolves," explains Nestle. "But in patients with psoriasis, it's stuck in this higher level of equilibrium."

Nestle's model also shows how the production of cytokines in patients can deviate from its stable state, triggering oscillations that might lead to flare ups — even in the absence of an environmental trigger. This could explain some of the differences in skin symptoms observed among patients, an idea that biologists can now explore in the lab. These models are "a novel way to create testable hypotheses", says Nestle.

REVEALING SKIN

One intriguing finding from recent studies of skin immunology is the extent to which psoriasis shares underlying mechanisms with other inflammatory conditions (see 'Deep exploration', page S56). Nestle's team has found[5] that people with psoriasis have a variant in the gene coding for a receptor for the interleukin-23 cytokine (IL-23). This same variant has also been found in Crohn's disease, a form of inflammatory bowel disease, and ankylosing spondylitis, a painful, chronic inflammation of the spinal joints. "Psoriasis is a window into other chronic inflammatory diseases," says Nestle.

Studying psoriasis as a model for chronic inflammation has a number of advantages. One is that the skin is readily accessible. Another is that psoriatic lesions are well defined and accessible: they can be photographed and biopsied, so responses to experimental therapies are easier to quantify than those for internal diseases such as rheumatoid arthritis. As a result, psoriasis is becoming a proof-of-principle testing ground for new targeted drugs.

In the immediate future, immunologists face the challenge of navigating the deluge of data in order to unravel the remaining mysteries about the role of the immune system in psoriasis. Many of the studies conducted so far focus on established disease. Much less is known about what causes the faulty immune reaction in the first place. What is the molecular trigger for the condition? To what extent are the molecular mechanisms behind psoriasis shared with other conditions? And can the system ever be properly reset?

Krueger believes we are only beginning to reveal the skin's dynamic role. "There's a tremendous amount to be learned," he says, "not only about psoriasis, but also about skin immunology." There is a long way to go in the skin's scientific makeover. ■

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