A 24-year-old woman undergoes medical evaluation for kidney failure and epilepsy-like convulsions that fail to respond to antiepileptic drugs. Her most visible sign of illness, though, is a red rash extending over the bridge of her nose and onto her cheeks, in a shape resembling a butterfly.

A 63-year-old woman insists on hospitalization to determine why she is fatigued, her joints hurt, and breathing sometimes causes sharp pain. Ever since her teen years she has avoided the sun, which raises painful blistering rashes wherever her skin is unprotected.

A 20-year-old woman is surprised to learn from a routine health exam that her urine has an abnormally high protein level—a sign of disturbed kidney function. A renal biopsy reveals inflammation.

Although the symptoms vary, the underlying disease in all three patients is the same—systemic lupus erythematosus, which afflicts an estimated 1.4 million Americans, including one out of every 250 African-American women aged 18 to 65. It may disrupt almost any part of the body: skin, joints, kidneys, heart, lungs, blood vessels or brain. At times, it becomes life-threatening.

Scientists have long known that, fundamentally, lupus arises from an immunological malfunction involving antibody molecules. The healthy body produces antibodies in response to invaders, such as bacteria. These antibodies latch onto specific molecules that are sensed as foreign (antigens) on an invader and then damage the interloper directly or mark it for destruction by other parts of the immune system. In patients with lupus, however, the body produces antibodies that perceive its own molecules as foreign and then launch an attack targeted to those “self-antigens” on the body’s own tissues.

Self-attack—otherwise known as autoimmunity—is thought to underpin many diseases, including type 1 diabetes, rheumatoid arthritis, multiple sclerosis and, possibly, psoriasis. Lupus, however, is at an extreme. The immune system reacts powerfully to a surprising variety of the patient’s molecules, ranging from targets exposed at the surface of cells to some inside of cells to even some within a further sequestering chamber, the cell nucleus. In fact, lupus is notorious for the presence of antibodies that take aim at the patient’s DNA. In the test tube, these anti-DNA “autoantibodies” can directly digest genetic material.

Until recently, researchers had little understanding of the causes of this multipronged assault. But clues from varied lines of research are beginning to clarify the underlying molecular events. The work is also probing the most basic, yet still enigmatic, facets of immune system function: the distinction of self from nonself; the maintenance of self-tolerance (nonaggression against native tissues); and the control over the intensity of every immune response. The discoveries suggest tantalizing new means of treating or even preventing not only lupus but also other autoimmune illnesses.

Some Givens

One thing about lupus has long been clear: the autoantibodies that are its hallmark contribute to tissue damage in more than one way. In the blood, an autoantibody that recognizes a particular self-antigen can bind to that antigen, forming a so-called immune complex, which can then deposit itself in any of various tissues. Autoantibodies can also recognize self-antigens already in tissues and generate immune complexes on-site. Regardless of how the complexes accumulate, they spell trouble.

TAMING LUPUS

LUPUS, technically lupus erythematosus, means “the red wolf.” It was so named because a face rash particular to the disorder often makes people look wolflike.
**Overview/Lupus**

- Lupus arises when the immune system mistakenly produces antibodies that attack the body’s own tissues, including the kidneys, skin and brain.
- The causes of this attack are complex, but a central component seems to be aberrant signaling within and between at least two types of immune cells: B lymphocytes (the antibody producers) and the T lymphocytes that help to activate the B cells.
- Several drugs under study aim to protect tissues by normalizing such signaling and quelling abnormal antibody production.

**PATHWAYS TO DISEASE**

The processes underlying lupus are known in a general way (flowchart). But many of the details remain murky, such as how hormones and environmental triggers precipitate immune attacks on the body’s tissues.

For one, they tend to recruit immune system entities known as complement molecules, which can directly harm tissue. The complexes, either by themselves or with the help of the complement molecules, also elicit an inflammatory response. This response involves an invasion by white blood cells that attempt to wall off and destroy any disease-causing agents. Inflammation is a protective mechanism, but if it arises in the absence of a true danger or goes on for too long, the inflammatory cells and their secretions can harm the tissues they are meant to protect. Inflammation can additionally involve the abnormal proliferation of cells native to an affected tissue, and this cellular excess can disrupt the normal functioning of the tissue. In the kidney, for instance, immune complexes can accumulate in glomeruli, the organ’s blood-filtering knots of capillary loops. Excessive deposition then initiates glomerulonephritis, a local inflammatory reaction that can lead to kidney damage.

Beyond inciting inflammation, certain lupus autoantibodies do harm directly. In laboratory experiments, they have been shown to bind to and then penetrate cells. There they become potent inhibitors of cellular function.

The real mystery about lupus is what precedes such events. Genetic predisposition seems to be part of the answer, at least in some people. About 10 percent of patients have close blood relatives with the disease, a pattern that usually implies a genetic contribution. Moreover, investigators have found greater lupus concordance—either shared lupus or a shared absence of it—in sets of identical twins (who are genetically indistinguishable) than in sets of fraternal twins (whose genes generally are no more alike than those of other pairs of siblings).

**Genetic Hints**

Spurred by such findings, geneticists are hunting for the genes at fault, including those that confer enhanced susceptibility to the vast majority of patients who have no obvious family history of the disease. Knowledge of the genes, the proteins they encode and the normal roles of these proteins should one day help clarify how lupus develops and could point to ways to better control it.

In mice prone to lupus, the work has identified more than 30 fairly broad chromosomal regions associated to some extent either with lupus or with resistance to it. Some are tied to specific elements of the disease. One region, for example, apparently harbors genes that participate in producing autoantibodies that recognize components of the cell nucleus (although the region itself does not encode antibodies); another influences the severity of the kidney inflammation triggered by lupus-related immune complexes.

In human lupus, the genetic story may be even more mind-boggling. An informative approach scans DNA from families with multiple lupus patients to identify genetic features shared by the patients but not by the other family...
members. Such work has revealed a connection between lupus and 48 chromosomal regions. Six of those regions (on five different chromosomes) appear to influence susceptibility most. Now investigators have to identify the lupus-related genes within those locales.

Already it seems fair to conclude that multiple human genes can confer lupus susceptibility, although each gene makes only a hard-to-detect contribution on its own. And different combinations of genes might lay the groundwork for lupus in different people. But clearly, single genes are rarely, if ever, the primary driver; if they were, many more children born to a parent with lupus would be stricken. Lupus arises in just about 5 percent of such children, and it seldom strikes in multiple generations of a family.

**Many Triggers**

If genes alone rarely account for the disease, environmental contributors must play a role. Notorious among these is ultraviolet light. Some 40 to 60 percent of patients are photosensitive: exposure to sunlight, say for 10 minutes at midday in the summer, may suddenly cause a rash. Prolonged exposure may also cause flares, or increased symptoms. Precisely how it does so is still unclear. In one scenario, ultraviolet irradiation induces changes in the DNA of skin cells, rendering the DNA molecules alien (from the viewpoint of the body’s immune defenses) and thus potentially antigenic. At the same time, the irradiation makes the cells prone to breakage, at which point they will release the antigens, which can then provoke an autoimmune response.

Environmental triggers of lupus also include certain medications, among them hydralazine (for controlling blood pressure) and procainamide (for irregular heartbeat). But symptoms usually fade when the drugs are discontinued. In other cases, an infection, mild or serious, may act as a lupus trigger or aggravator. One suspect is Epstein-Barr virus, perhaps best known for causing infectious mononucleosis, or “kissing disease.” Even certain vaccines may provoke a lupus flare. Yet despite decades of research, no firm proof of a bacterium, virus or parasite that transmits lupus has been put forth. Other possible factors include diets high in saturated fat, pollutants, cigarette smoking, and perhaps extreme physical or psychological stress.

**Perils of Cell Suicide**

Another line of research has revealed cellular and molecular abnormalities that could well elicit or sustain autoimmune activity. Whether these abnormalities are usually caused more by genetic inheritance or by environmental factors remains unknown. People may be affected by various combinations of influences.

One impressive abnormality involves a process known as apoptosis, or cell suicide. For the body to function properly, it has to continually eliminate cells that have reached the end of their useful life or turned dangerous. It achieves this pruning by inducing the cells to make proteins that essentially destroy the cell from within—such as by hacking to pieces cellular proteins and the chromosomes in the nucleus. But the rate of apoptosis in certain cells—notably, the B and T lymphocytes of the immune system—is excessive in those who have lupus.

When cells die by apoptosis, the body usually disposes of the remains efficiently. But in those with lupus, the disposal system seems to be defective. This double whammy of increased apoptosis and decreased clearance can promote autoimmunity in a fairly straightforward way: if the material inside the apoptotic cells is abnormal, its ejection from the cells in quantity could well elicit the production of antibodies that mistakenly perceive the aberrant material as a sign of invasion by a disease-causing agent. And such antibody production is especially likely if the ejected material, rather than being removed, accumulates enough to call attention to itself.

Unfortunately, the material that spills from apoptotic cells of those with lupus, especially the chromosomal fragments, is often abnormal. In healthy cells, certain short sequences of DNA carry methyl groups that serve as tags controlling gene activity. The DNA in circulating immune complexes from lupus patients is undermethylated. Scientists have several reasons to suspect that this methylation pattern might contribute to autoimmunity. In the test tube, abnormally methylated DNA can stimulate a number of cell types involved in immunity, including B lymphocytes, which, when mature, become antibody-spewing factories. (Perhaps the body misinterprets these improperly methylated stretches as a sign that a disease-causing agent is present and must be eliminated.) Also, certain drugs known to cause lupus symptoms lead to undermethylation of DNA in T cells, which leads to T cell autoreactivity in mice.

All in all, the findings suggest that apoptotic cells are a potential reservoir of autoantigens that are quite capable of provoking an autoantibody response. In further support of this idea, intravenous
Some Treatment Strategies under Study

<table>
<thead>
<tr>
<th>TYPE OF AGENT</th>
<th>STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocker of B7’s interaction with CD28, to impede activation of helper T cells</td>
<td>Immune Tolerance Network, a research consortium, and the National Institutes of Health are undertaking a small human trial of a blocker called RG2077</td>
</tr>
<tr>
<td>Blocker of BAFF’s interaction with its receptor, to keep BAFF (also called BLyS) from promoting B cell survival and antibody production</td>
<td>Human Genome Sciences (Rockville, Md.) is evaluating one such drug, LymphoStat-B, in a multicenter trial; ZymoGenetics (Seattle) and Serono S.A. (Geneva, Switzerland) are conducting an early human trial of an agent named TACI-Ig</td>
</tr>
<tr>
<td>Blocker of B cell receptors and of antibodies that recognize the body’s own DNA, to inhibit the production and activity of antibodies that target such DNA</td>
<td>La Jolla Pharmaceuticals (San Diego) is conducting a multicenter trial of abetimus sodium (Riquent) against lupus-related kidney disease</td>
</tr>
<tr>
<td>Antibody to CD20, to deplete B cells</td>
<td>Genentech (South San Francisco, Calif.) and Biogen Idec (Cambridge, Mass.) are conducting a multicenter lupus trial of rituximab (Rituxan), a drug already approved for B cell cancer</td>
</tr>
<tr>
<td>Complement inhibitor, to prevent complement-mediated tissue damage</td>
<td>Alexion Pharmaceuticals (Cheshire, Conn.) found evidence of disease amelioration in mice given an inhibitor of complement C5</td>
</tr>
</tbody>
</table>
Deranged Cells

The problem mostly seems to stem from signaling imbalances within B cells. In the healthy body, a B cell matures into an antibody-secreting machine—known as a plasma cell—only after antibodylike projections on the B cell’s surface (B cell receptors) bind to a foreign antigen. If a B cell instead attaches to a self-component, this binding normally induces the cell to kill itself, to retreat into a nonresponsive (anergic) state or to “edit” its receptors until they can no longer recognize the self-antigen.

Whether the cell responds appropriately depends in large measure on the proper activity of the internal signaling pathways that react to external inputs. Mouse studies show that even subtle signaling imbalances can predispose animals to produce antibodies against the self. And various lines of evidence indicate that certain signaling molecules (going by such names as Lyn, CD45 and SHP-1) on and in B cells of patients with lupus are present in abnormal amounts.

Other work suggests that it is not only the B cells that are deranged. For a B cell to become an antibody maker, it must do more than bind to an antigen. It must also receive certain stimulatory signals from immune system cells known as helper T lymphocytes. Helper cells of lupus patients are affected by signaling abnormalities reminiscent of those in the B cells. The T cell aberrations, though, may lead to autoantibody production indirectly—by causing the T cells to inappropriately stimulate self-reactive B cells.

All theorizing about the causes of lupus must account not only for the vast assortment of autoantibodies produced by patients but also for another striking aspect of the disease: the disorder is 10 times as common in women than in men. It also tends to develop earlier in women (during childbearing years). This female susceptibility—a pattern also seen in some other autoimmune diseases—may stem in part from greater immune reactivity in women. They tend to produce more antibodies and lymphocytes than males and, probably as a result, to be more resistant to infections. Among mice, moreover, females reject foreign grafts more rapidly than the males do. Perhaps not surprisingly, sex hormones seem to play a role in this increased reactivity, which could explain why, in laboratory animals, estrogens exacerbate lupus and androgens ameliorate it.

Estrogens could pump up immune reactivity in a few ways. They augment the secretion of prolactin and growth hormone, substances that contribute to the proliferation of lymphocytes, which bear receptor molecules sensitive to estrogens. Acting through such receptors, estrogens may modulate the body’s immune responses and may even regulate lymphocyte development, perhaps in ways that impair tolerance of the self.

Toward New Therapies

Those of us who study the causes of lupus are still pondering how the genetic, environmental and immunological features that have been uncovered so far collaborate to cause the disease. Which events come first, which are most important, and how much do the underlying processes differ from one person to another? Nevertheless, the available clues suggest at least a partial scenario for how the disease could typically develop.

The basic idea is that genetic susceptibility and environmental influences may share responsibility for an impairment of immune system function—more specifically an impairment of the signaling within lymphocytes and possibly within other cells of the immune system, such as those charged with removing dead cells and debris. Faulty signaling, in turn, results in impaired self-tolerance, accelerated lymphocyte death, and defective disposal of apoptotic cells and administration of large quantities of irradiated apoptotic cells is able to induce autoantibody synthesis in normal mice.

Hence, part of the underlying process leading to the formation of destructive immune complexes may involve the body’s production of foreign-seeming antigens, which cause the body to behave as if tissues bearing those antigens were alien and threatening. But other work indicates that, in addition, the B lymphocytes of lupus patients are inherently deranged; they are predisposed to generate autoantibodies even when the self-molecules they encounter are perfectly normal. In other words, the mechanisms that should ensure self-tolerance go awry.

MONCEF ZOUALI, an immunologist and molecular biologist, is a director of research at INSERM, the French national institute for medical research. He focuses on conducting basic research into the molecular causes of systemic autoimmune diseases and on translating scientific insights into useful approaches to disease management. Zouali has edited several books on autoimmunity and won research awards.
Physicians who suspect that a patient, whether female or male, has lupus continue to be hampered by the lack of a conclusive test. Because immunological self-attack may underlie many illnesses, even a classic sign of lupus—the presence of antinuclear autoantibodies—does not unmistakably diagnose this disorder.

In the absence of a sure test, doctors might gather information from a variety of sources, including not only laboratory tests but also the patient’s description of current symptoms and medical history. To assist, the American College of Rheumatology has issued a list of 11 criteria that could indicate lupus. Seven concern symptoms, such as arthritis, sensitivity to sunlight or a butterfly facial rash. (The butterfly pattern is still unexplained.) The other four describe laboratory findings that include the presence of antinuclear autoantibodies or depressed concentrations of lymphocytes.

Researchers will consider a subject to have lupus if the person meets four of the criteria, but physicians might base a diagnosis on fewer cues, especially if a patient has strong indicators of the disorder, such as clinical evidence of abnormalities in several different organ systems combined with the presence of antinuclear autoantibodies. For more on common manifestations of lupus, visit the Lupus Foundation of America: www.lupus.org/ or the Lupus site: www.uklupus.co.uk/  —M.Z.

**Current Criteria**

- **Malar rash** (a rash, often butterfly-shaped, over the cheeks)
- **Discoid rash** (a type involving red raised patches)
- **Photosensitivity** [reaction to sunlight in which a skin rash arises or worsens]
- **Nose or mouth ulcers, typically painless**
- **Nonerosive arthritis** [which does not involve damage to the bones around the joints] in two or more joints
- **Inflammation of the lining in the lung or heart** [also known as pleuritis or pericarditis]
- **Kidney disorder** marked by high levels in the urine of protein or of abnormal substances derived from red or white blood cells or kidney tubule cells
- **Neurological disorder** marked by seizures or psychosis not explained by drugs or metabolic disturbances [such as an electrolyte imbalance]
- **Blood disorder** characterized by abnormally low concentrations of red or white blood cells or platelets [specifically, hemolytic anemia, leukopenia, lymphopenia or thrombocytopenia] and not caused by medications
- **Positive test for antinuclear antibodies [ANA]** not explained by drugs known to trigger their appearance
- **Positive test for antibodies against double-stranded DNA or certain phospholipids** or a false positive result on a syphilis test

The self-antigens they release. Abundantly available to the body’s unbalanced immune surveillance, the antigens then misdirect the immune system, inducing it to attack the self.

Drugs do exist for lupus, but so far they focus on dampening the overall immune system. In other words, they are nonspecific: instead of targeting immunological events underlying lupus in particular, they dull the body’s broad defenses against infectious diseases. Corticosteroids, for instance, reduce inflammation at the cost of heightening susceptibility to infections.

The challenge is to design new drugs that prevent autoimmune self-attacks without seriously hobbling the body’s ability to defend itself against infection. To grasp the logic of the approaches being attempted, it helps to know a bit more about how helper T cells usually abet the transformation of B cells into vigorous antibody makers [see box on pages 74 and 75].

First, the helper cells themselves must be activated, which occurs through interactions with so-called professional antigen-presenting cells (such as macrophages and dendritic cells). These antigen presenters ingest bacteria, dead cells and cellular debris, chop them up, join the fragments to larger molecules (called MHC class II molecules) and display the resulting MHC-antigen complexes on the cell surface. If the receptor on a helper T cell recognizes a complex and binds to it, the binding conveys an antigen-specific signal into the T cell. If, at the same time, a certain T cell projection near the receptor links to a particular partner (known as a B7 molecule) on the antigen-presenting cell, this binding will convey an antigen-independent, or co-stimulatory, signal into the T cell. Having received both messages, the T cell will switch on; that is, it will produce or display molecules needed to activate B cells and will seek out those cells.

Like the professional antigen-presenting cells, B cells display fragments of ingested material—notably fragments of an antigen they have snared—on MHC class II molecules. If an activated helper T cell binds through its receptor...
to such a complex on a B cell, and if the T and B cells additionally signal each other through co-stimulatory surface molecules, the B cell will display receptors for small proteins called cytokines. These cytokines, which are secreted by activated helper T cells, induce the B cell to proliferate and mature into a plasma cell, which dispatches antibodies that specifically target the same antigen recognized by the coupled B and T cells.

Of course, any well-bred immune response shuts itself off when the danger has passed. Hence, after an antigen-presenting cell activates a helper T cell, the T cell also begins to display a “shutoff” switch known as CTLA-4. This molecule binds to B7 molecules on antigen-presenting cells so avidly that it links to most or even all of them, thereby putting a break on any evolving helper T and, consequently, B cell responses.

One experimental approach to treating lupus essentially mimics this shutoff step, dispatching CTLA-4 to cap over B7 molecules. In mice prone to lupus, this method prevents kidney disease from progressing and prolongs life. This substance is beginning to be tested in lupus patients; in those with psoriasis, initial clinical trials have shown that the treatment is safe.

A second approach would directly impede the signaling between helper T cells and B cells. The T cell molecule that has to “clasp hands” with a B cell molecule to send the needed co-stimulatory signal into B cells is called CD154. The helper cells of lupus patients show increased production of CD154, and in mice prone to the disease, antibodies engineered to bind to CD154 can block B cell activation, preserve kidney function and prolong life. So far early human tests of different versions of anti-CD154 antibodies have produced a mixture of good news and bad. One version significantly reduced autoantibodies in the blood, protein in the urine and certain symptoms, but it also elicited an unacceptable degree of blood-clot formation. A different version did not increase thrombosis but worked poorly. Hence, no one yet knows whether this approach to therapy will pan out.

A third strategy would interfere with B cell activity in a different way. Certain factors secreted by immune system cells, such as the cytokine BAFF, promote cell survival after they bind to B cells. These molecules have been implicated in various autoimmune diseases, including lupus and its flares: mice genetically engineered to overproduce BAFF or one of its three receptors on B cells develop signs of autoimmune disease, and BAFF appears to be overabundant both in lupus-prone mice and in human patients. In theory, then, preventing BAFF from binding to its receptors should minimize antibody synthesis. Studies of animals and humans support this notion. In mice, a circulating decoy receptor designed to mop up BAFF before it can find its true receptors, alleviates lupus and lengthens survival. Findings for a second decoy receptor are also encouraging. Human trials are in progress.

Targeting other cytokines might help as well. Elevated levels of interleukin-10 and depressed amounts of transforming growth factor beta are among the most prominent cytokine abnormalities reported in lupus, and lupus-prone mice appear to benefit from treatments that block the former or boost the latter. Taking a different tack, investigators studying various autoimmune conditions are working on therapies aimed specifically at reducing B cell numbers. An agent called rituximab, which removes B cells from circulation before they are able to secrete antibodies, has shown promise in early trials in patients with systemic lupus.

Some other therapies under investigation include molecules designed to block production of anti-DNA autoantibodies or to induce those antibodies to bind to decoy compounds that would trap them and provoke their degradation. An example of such a decoy is a complex consisting of four short DNA strands coupled to an inert backbone. Although the last idea is intriguing, I have to admit that the effects of introducing such decoys are apt to be complex.

Certain cytokines might be useful as therapies, but these and other protein drugs could be hampered by the body’s readiness to degrade circulating proteins. To circumvent such problems, researchers are considering gene therapies, which would give cells the ability to produce useful proteins themselves. DNA encoding transforming growth factor beta has already been shown to treat lupus in mice, but too few tests have been done yet in humans to predict how useful the technique will be in people. Also, scientists are still struggling to perfect gene therapy techniques in general.

As treatment-oriented investigators pursue new leads for helping patients, others continue to probe the central enigmas of lupus. What causes the aberrant signaling in immune cells? And precisely how does such deranged signaling lead to autoimmunity? The answers may well be critical to finally disarming the body’s mistaken attacks on itself.

MORE TO EXPLORE


www.lupusresearch.org