Bdellovibrio's voracious appetite for bacteria has been known for more than 40 years, and it is puzzling to Schuster that the subject was not more vigorously pursued by researchers. He has already started cooperating with a biotechnology company to develop a treatment for external wounds infected by *Pseudomonas*. "So far we're concentrating on external applications," he said, but he is disappointed in the low level of interest from industry, which considers the discovery to be too far from application for investment. Meanwhile, the sequencing of another *Bdellovibrio* strain is allowing genomic comparisons, further increasing the understanding of the genes involved in predation. These number over 240. Not surprisingly, "genetic modification [of *Bdellovibrio*] is still a thing of the future; we need to understand the genetic traits first," Schuster commented. But the basic research is already a perfect example of the confluence of many fields: microbiology, cell biology, molecular biology and immunology.

Schuster already thinks that his potential living antibiotic stands a better chance of success than a much older field of research involving bacteriophages. These bacterium-infecting viruses, he says, have a much higher mutation rate and would therefore be less stable in applications. Phages have evolved specifically to attack only bacteria, and are very selective about their prey species. But given that as long ago as the 1930s, a US pharmaceutical company included phages in its list of biological therapies, one cannot help but wonder why they have not yet made it into widespread use. And it was not as if the company was pushing some kind of alchemist's panacea: the curative powers of phages had first been observed by researchers at the Pasteur Institute in Paris in 1917, where they were administered to sufferers of dysentery and several other bacterial infections, with remarkable results. The broad-spectrum efficacy of penicillin, first used in the 1940s, however, eclipsed these minute killing machines.

The specificity of phages, as with many organism-based strategies, precludes their use as a broad-spectrum antibiotic; the disease-causing pathogen must first be accurately identified before treatment. But this is also their advantage: unlike broad-spectrum antibiotics, which not only simultaneously breed resistance in many

bacteria, but also wipe out friendly commensals, a particular phage delicately picks off only the bacterial species that it naturally infects. That may make phages the doctor's friend, but does not necessarily interest large pharmaceutical companies. Biotech companies, on the other hand, see plenty of fresh pickings, as Glenn Morris, co-founder of Intralytix Inc. (Baltimore, MD, USA) and Chairman of the Department of Epidemiology and Preventive Medicine at the University of Maryland School of Medicine (also in Baltimore) agrees. He thinks the time is ripe for phages to make it to market. Commenting on the abrupt decline in phage preparations in the 1940s, he said, "there were some problems with initial phage preparations because they weren't pure, and frequently [the medics] did not know what they were treating." That has all changed: "[today] you're talking about the sophistication that comes with a further 60 years of science," he added.

Intralytix Inc. has already performed trials of phages against vancomycin-resistant enterococci in animal models, showing the phages to be "very safe, and very efficacious" in the words of Morris. Multidrug-resistant *Pseudomonas* is also on the hit list

of the phage therapy developers, and Morris is confident, despite Schuster's scepticism, that here too a treatment for humans is not far off. Given that a phage is a much simpler biological entity than a bacterium, he is sure of who the winner will be: "It's going to be tough enough to get approval for phage use in medications; I'm not sure I'd like to take a *Bdellovibrio* through an FDA approval process." Although Morris concedes that phages will not be a cure-all, this is precisely their strength: in combination with traditional antibiotics, they will probably contribute to new antibiotic strategies that keep pathogens on their toes more than antibiotics alone.

Developing the antibiotics of the future is not really about whether bacteria, phages or chemical antibiotics will win. The combination of increasing pressure to attack infectious disease with new and diverse strategies, and a market sector that takes risks and is content with smaller profits than the pharmaceutical giants, will probably generate a range of novel biological products. Nature has, after all, done half the work for us.

Andrew Moore

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When the immune system goes on the attack

Thanks to advances in research, it may soon be easier to diagnose autoimmune diseases earlier, but therapy remains a tricky problem

Eileen O'Connor of New York City had been suffering for more than three years and neither she—a nurse, healthcare lawyer and epidemiologist—nor numerous specialists could make sense of her symptoms: persistent respiratory infections, a collapsed lung, difficulty breathing, repeated falls, broken bones and torn cartilage. Only recently did she learn that she has systemic lupus erythematosus (SLE), an elusive autoimmune disease (AD) that affects the lungs, muscles, brain, heart and kidneys. Explaining why it

was so difficult to diagnose, Joan Merrill—Professor of Medicine at the University of Oklahoma's Health Sciences Center (Oklahoma City, OK, USA)—said, "lupus shows extreme variability in what organ or organs it attacks."

Like lupus, many ADs are difficult to diagnose and treat. Some affect multiple organ systems, whereas others, such as rheumatoid arthritis (RA), antibody-mediated thrombosis, Sjögren's syndrome, myasthenia gravis, type 1 diabetes and Graves' disease, target primarily one organ, although

they can also wreak havoc in other organs, including the pancreas (arthritis) and the heart (hyperthyroidism, diabetes and RA). And truly effective treatment is available for only a few diseases, such as Graves' disease. "For most of these diseases, the concept of remission does not even exist," commented Richard Burt, Associate Professor of Medicine at the Feinberg School of Medicine, Northwestern University (Chicago, IL, USA).

The common characteristic of all ADs is an immune system that attacks healthy tissues, thus provoking inflammation, as well as tissue and organ damage. Autoimmunity is present to some extent in everyone, but it usually causes no harm. ADs, however, progress to a pathogenic state that involves the whole immune system: antigens, antigen-presenting cells, T and B lymphocytes, messenger molecules, cytokines, chemokines and their receptors, and signalling and co-stimulatory molecules (Mackay, 2000). There are an estimated 80 ADs, which affect 14–22 million

Americans, or 5%–8% of the population, and their prevalence is on the rise, according to the US National Institutes of Health (NIH). ADs affect a disproportionate number of women—the female to male ratio is 50:1 in Hashimoto's syndrome (hypothyroiditis), 9:1 in lupus, primary biliary cirrhosis and antiphospholipid syndrome, 7:1 in Graves' disease, 4:1 in RA and 2:1 in multiple sclerosis (MS) and myasthenia gravis. Others strike particular ethnic groups more frequently: lupus is three times as common in African Americans and Latinos/Hispanics, whereas MS and type 1 diabetes are seen more frequently in Caucasians. "The predominance of AD

among women suggests that sex hormones may modulate susceptibility," according to Caroline Whitacre, Professor and Chair of Molecular Virology, Immunology and Medical Genetics at Ohio State University (Columbus, OH, USA).

The central role of sex hormones is quite obvious, as they modulate T-cell receptor signalling, activation of cytokine genes and lymphocyte homing. Ongoing research by Betty Diamond at the Albert

"For most of these diseases, the concept of remission does not even exist"

ADs run in families: a mother may have rheumatoid arthritis, her sister psoriasis and a daughter lupus. And patients afflicted with one organ-specific AD are often subsequently diagnosed with another.

Infectious diseases also seem to have a significant role in the pathogenesis of ADs. Immune cells responding to infection can cross-react with normal tissues, leading to an autoimmune response, explained Michael Oldstone, head of the Viral Immunobiology Laboratory at the Scripps Research Institute (La Jolla, CA, USA). "In molecular mimicry, a small number of self-reactive T-cells are expanded with cross-reactive epitopes to produce a quantity of T-cells sufficient to create disease," he said. "The initial insult is often an infection, which affects a target tissue, such as the brain or pancreas, but does not

cause disease. The infection may be cleared, but when the insult is repeated, self-reactive cells are expanded from a few autoreactive cells." Oldstone's work links lymphocytic choriomeningitis virus with type 1 diabetes. Other researchers are investigating the role of human herpes virus 6 and Epstein-Barr virus in MS, *Helicobacter pylori* in gastric autoimmunity (Amedei *et al*, 2003) and *Listeria* and *Mycobacterium avium* in Crohn's disease. Noel Rose, Professor at Johns Hopkins University's Bloomberg School of Public Health (Baltimore, MD, USA) and Director of its Center for Autoimmune Disease Research, is studying how group

Einstein College of Medicine (Bronx, NY, USA) indicates that oestrogen may in fact predispose women to SLE by reducing B-cell tolerance and dampening apoptotic processes (Bynoe *et al*, 2000). A new study (Kramer *et al*, 2004) from the Baylor College of Dentistry (Dallas, TX, USA) showed that decreasing oestrogen levels set off a chain reaction of inflammation. Research on knockout mice also demonstrated that the oestrogen receptor mediates a variety of ADs (Liu *et al*, 2003). In addition, genetic studies have revealed that different ADs may share the same susceptibility genes, such as lupus and RA (Helms *et al*, 2003; Tokuhiko *et al*, 2003). In fact,

B coxsackieviruses induce autoimmune myocarditis in genetically predisposed mice. He identified key cytokines produced during viral infection that determine the development of autoimmunity and showed that blocking these prevents autoimmune myocarditis.

Treating ADs remains a delicate balancing act. "Two approaches are possible with ADs: slightly dampening down the entire immune system, and targeting one part of it," said Merrill. "Probably, the optimal approach would be combining both types of treatments." Until the last decade, the major weapons against an overactive immune system were steroids, chemotherapy and major immunosuppressants—very blunt instruments with serious side effects. Better treatments come from the discovery of new targets and the development of monoclonal antibodies, antisense RNA and other drugs that target only parts of the immune system by blocking specific molecules in the inflammatory pathway. The first generation of these therapeutics, primarily directed against cytokines, are monoclonal antibodies. Amgen's (Thousand Oaks, CA, USA) ENBREL® (etanercept), Centocor's (Malvern, PA, USA) Remicade® (infliximab) and Abbott Laboratories' (Abbott Park, IL, USA) HUMIRA® (adalimumab), which interfere with the pro-inflammatory tumour necrosis factor- α , have already become bestsellers for treating RA. Amgen's Kineret® (anakinra) is an antagonist to the interleukin-1 receptor, another pro-inflammatory cytokine. These drugs avoid some of the more serious side effects that are associated with steroids, but most still increase the risk of infections.

Treating ADs remains a delicate balancing act

Other specific drug targets include adhesion molecules, which promote T-cell migration, aggregation and activation, ICE (interleukin- β -converting enzyme) and cytotoxic-T-lymphocyte-associated antigen 4 immunoglobulin (CTLA4-Ig), a T-cell regulatory protein that acts as an 'off' switch for the whole immune system. Protein Design Labs' (Fremont, CA, USA) Nuvion® (visilizumab) targets the CD3 receptor on T cells to treat psoriasis. The

Immune Response Corp. (Carlsbad, CA, USA) is developing a vaccine for MS, NeuroVax™, which downregulates pathogenically activated T cells. Antisense drugs in development include a candidate for treating psoriasis by inhibiting insulin-like growth factor 1 receptor (IGF1r; Antisense Therapeutics Ltd, Toorak, Victoria, Australia), and Isis' (Carlsbad, CA, USA) inhibitor of CD49d, which prevents white blood cells from leaving the blood and entering the central nervous system, to stop the progression of MS.

...work to identify, prevent, treat or even reverse an autoimmune disease is most advanced for type 1 diabetes, because the main culprits ... have been known for a long time

As many ADs share underlying immune system defects, various drugs are also being tested for different diseases: Remicade, used by half a million people for RA in the USA, is now in trials for Crohn's disease, and Enbrel was recently approved in the USA for treating psoriasis. Biogen Idec's (Cambridge, MA, USA) and Elan's (Dublin, Ireland) Antegren® (natalizumab), a selective adhesion molecule inhibitor designed to inhibit certain immune cells from migrating into chronically inflamed tissue, is being tested for MS, Crohn's disease and RA, and Nuvion is being tested for severe ulcerative colitis. This June, a British–Polish study showed that Rituxan® (rituximab), an antibody developed by Genentech (San Francisco, CA, USA) to treat lymphoma, is also effective against RA (Edwards *et al*, 2004).

Equally important is improving diagnosis, because early identification of susceptible patients may help to treat them and prevent disease progression before the onset of symptoms. A recent study (Scofield, 2004) showed that it might indeed be possible to predict ADs years before illness appears by measuring auto-antibodies. Hal Scofield, Professor of Medicine at the University of Oklahoma's Health Sciences Center, reviewed the blood samples of six million US military personnel, taken routinely on induction and then every two years after that. He traced the existence and persistence of

various auto-antibodies over a decade, and correlated them to individuals who ultimately received lupus diagnoses. "Antinuclear antibodies and anti-Ro appeared as early as 10 years before first onset of [SLE] disease," Scofield said. "Except in diabetes, it had not been shown before that the respective auto-antibodies preceded the emergence of disease." He also cited other studies showing that the existence of other antibodies can predict who will develop RA, primary biliary cirrhosis, autoimmune thyroid disease and type 1 diabetes, a median of 4–5 years before disease onset. "If you can identify a patient before he becomes ill, it may be possible to use an immunomodulatory strategy to prevent him from becoming ill," Scofield said.

Such work to identify, prevent, treat or even reverse an autoimmune disease is most advanced for type 1 diabetes, because the main culprits—the antibodies targeting the insulin-producing β -cells—have been known for a long time. Several current studies examine auto-antibodies that appear in at-risk individuals before diabetes develops. Two NIH-sponsored trials will investigate the immune and metabolic events leading to disease onset in individuals with certain antibodies, and try to stop or delay the destruction of β -cells using drugs that have been approved to prevent organ rejection (www.diabetestrialnet.org). Others aim at specific antibodies. In 1993, Daniel Kaufman of the University of California Los Angeles's School of Medicine cloned the glutamic acid decarboxylase (GAD) gene and showed that a GAD vaccine could inhibit diabetes in mice with established autoimmune responses. On the basis of his work, Diamyd Medical (Stockholm, Sweden) is testing a drug against GAD antibodies. At the end of March, Diamyd reported data from a phase II trial showing that its drug increased insulin production in patients with a slowly progressing diabetes, called latent autoimmune diabetes. The next trial will treat new-onset type 1 diabetes, with the goal of arresting the destruction of remaining β -cells. Diabetogen (London, Ontario, Canada) is also developing a

But perhaps the best hope for long-term remissions in ADs is ... to 'reset' the whole immune system by replacing it with fresh cells

human T-cell-targeting monoclonal antibody with Abgenix (Fremont, CA, USA) to reverse disease onset.

Other advances aim to replace lost β -cells, including islet transplantation, implanting encapsulated porcine pancreatic β -cells (Microslet Inc., San Diego, CA, USA), transforming human fetal liver cells into insulin-producing cells, isolating pancreatic progenitor cells and transforming adult murine bone marrow cells to produce insulin. Last autumn, researchers at the Joslin Diabetes Center (Boston, MA, USA) even reversed autoimmune diabetes in mice by injecting spleen cells from a different mouse type together with an immunostimulatory drug (Kodama *et al*, 2003). But islet transplantation has had limited success due to a dearth of available organs and because immunosuppression that is used to induce tolerance to foreign cells actually kills those cells, according to Gordon Weir, Head of Islet Transplantation and Cell Biology at Joslin and Professor of Medicine at Harvard Medical School (Cambridge, MA, USA). Nevertheless, improvements are yielding results and about half of the recipients need no more insulin injections after their islet transplants (Robertson, 2004).

But perhaps the best hope for long-term remissions in ADs is coming from autologous haematopoietic stem-cell transplants (HSCTs) that 'reset' the whole immune system by replacing it with fresh cells. Richard Burt got this idea

14 years ago when working with cancer patients. "I noticed that patients who had had bone marrow transplants had to be re-immunised for infectious diseases because they'd lost their immune memory," he said. So began a plan to regenerate a naive immune system from uncommitted, newly developing stem cells.

"The concept of autologous HSCT presumes that the autoimmune disease is environmentally induced and not a genetic stem cell defect," Burt said. As his goal is immune suppression rather than destruction of bone marrow, he uses drugs, not radiation, which has decreased the risk of mortality fourfold. Infusing stem cells after immune suppression also results in faster patient recovery. Burt began testing HSCT eight years ago, and first reported positive results in lupus, MS and RA in the late 1990s. In 2003, he started using HSCT on Crohn's disease, and other researchers have tested it in scleroderma and juvenile chronic arthritis. Altogether, about 600 HSCTs have been performed in Europe and Asia, and 209 in the USA. Overall, Burt sees more benefit in patients who are less severely affected by their disease. Furthermore, when patients do relapse after HSCT—as do about one-third of those with lupus—drugs usually restore remissions.

REFERENCES

Amedei A, Bergman MP, Appelmek BJ, Azzurri A, Benagiano M, Tamburini C, van der Zee R, Telford JL, Vandenbroucke-Grauls CM, D'Elia MM, Del Prete G (2003) Molecular mimicry between *Helicobacter pylori* antigens and H⁺, K⁺-adenosine triphosphatase in human gastric autoimmunity. *J Exp Med* **198**: 1147–1156

Bynoe MS, Grimaldi CM, Diamond B (2000) Estrogen up-regulates Bcl-2 and blocks tolerance induction of naive B cells. *Proc Natl Acad Sci USA* **97**: 2703–2708

Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, Stevens RM, Shaw T (2004) Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *New Engl J Med* **350**: 2572–2581

Helms C *et al* (2003) A putative RUNX1 binding site variant between *SLC9A3R1* and *NAT9* is associated with susceptibility to psoriasis. *Nat Genet* **4**: 349–356

Kodama S, Kühtreiber W, Fujimura S, Dale EA, Faustman DL (2003) Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science* **302**: 1223–1227

Kramer PR, Kramer SF, Guan G (2004) 17 β -estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. *Arthritis Rheum* **50**: 1967–1975

Liu HB, Loo KK, Palaszynski K, Ashouri J, Lubahn DB, Voskuhl RR (2003) Estrogen receptor α mediates estrogen's immune protection in autoimmune disease. *J Immunol* **171**: 6936–6940

Mackay IR (2000) Science, medicine and the future: tolerance and autoimmunity. *Br Med J* **321**: 93–96

Robertson RP (2004) Islet transplantation as a treatment for diabetes—a work in progress. *New Engl J Med* **350**: 694–705

Scofield RH (2004) Autoantibodies as predictors of disease. *Lancet* **363**: 1544–1546

Tokuhiro S *et al* (2003) An intronic SNP in a RUNX1 binding site of *SLC22A4*, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nat Genet* **4**: 341–348

Vicki Brower

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