Will you get sick?
Antibodies could foretell
the future of your health

Predicting Disease

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New PREDICTORS of Disease

Molecules called predictive autoantibodies appear in the blood years before people show symptoms of various disorders. Tests that detected these molecules could warn of the need to take preventive action.

By Abner Louis Notkins

A middle-aged woman—call her Anne—was taken aback when one day her right hand refused to hold a pen. A few weeks later her right foot began to drag reluctantly behind her left. After her symptoms worsened over months, she consulted a neurologist. Anne, it turned out, was suffering from multiple sclerosis, a potentially disabling type of autoimmune disease. The immune system normally jumps into action in response to bacteria and viruses, deploying antibodies, other molecules and various white blood cells to recognize and destroy trespassers. But in autoimmune disorders, components of the body’s immune system target one or more of the person’s own tissues. In Anne’s case, her defensive system had begun to turn against her nerves, eroding her ability to move.

Every story of autoimmune disease is sad—but collectively the impact of these illnesses is staggering. More than 40 autoimmune conditions have been identified, including such common examples as type 1 (insulin-dependent) diabetes, rheumatoid arthritis and celiac disease. Together they constitute the third leading cause of sickness and death after heart disease and cancer. And they afflict between 5 and 8 percent of the U.S. population, racking up an annual medical bill in the tens of billions of dollars.

Recent findings offer a way to brighten this gloomy picture. In the past 10 years a growing number of studies have revealed that the body makes certain antibodies directed against itself—otherwise known as autoantibodies—years, and sometimes a decade, before autoimmunity causes clinical disease, damaging tissues so much that people begin showing symptoms. This profound insight is changing the way that doctors and researchers think about autoimmune conditions and how long they take to arise. It suggests that physicians might one day screen a healthy person’s blood for certain autoantibodies and forecast whether a specific disease is likely to develop years down the line. Armed with such predictions, patients could start fighting the ailment with drugs or other available interventions, thereby preventing or delaying symptoms.

Those interventions may not be easy to find; most likely, preventive therapy would have to be tailored specifically for each condition. In certain disorders, such as myasthenia gravis, autoantibodies participate in the disease process, and so blocking the activity of the particular autoanti-
bodies at fault could be therapeutic. Autoantibodies that presage certain other conditions, though, probably are more sinister than fire, announcing brewing disease actually caused by other components of the immune system, such as cells known as T lymphocytes and macrophages. In those cases, preventive treatments would have to target the offending cells.

The revolution in predictive medicine and preventive care will take time and effort to effect. Many autoantibodies have been uncovered, but only a few large-scale trials have been conducted to evaluate how accurately they can predict disease. If inexpensive, quick tests for predictive autoantibodies can be developed, though, they could become as standard a part of routine checkups as cholesterol monitoring.

**Early Insight from Diabetes**

People familiar with advances in genetics might wonder why researchers would want to develop tests for predictive autoantibodies when doctors might soon be able to scan a person’s genes for those that put the individual at risk of various disorders. The answer is that most chronic diseases arise from a complex interplay between environmental influences and multiple genes (each of which makes but a small contribution to a disease). So detection of susceptibility genes would not necessarily reveal with any certainty whether or when an individual will come down with a particular autoimmune condition. In contrast, detection of specific autoantibodies would signal that a disease-causing process was already under way. Eventually, genetic screening for those with an inherited predisposition to a disease may help reveal those who need early autoantibody screening.

Studies of patients with type 1 diabetes provided the first clues that autoantibodies could be valuable for predicting later illness. In this condition, which typically arises in children or teenagers, the immune system ambushes the beta cells in the pancreas. These cells are the manufacturers of insulin, a hormone that enables cells to take up vital glucose from the blood for energy. When the body lacks insulin, cells starve and blood glucose levels soar, potentially leading to blindness, kidney failure, and a host of other complications.

Forty years ago type 1 diabetes was not yet recognized as an autoimmune disease, and no one knew what caused the beta cells to die. But in the 1970s Willy Gepts of Vrije University of Brussels in Belgium examined the pancreases of children who had died of the disease and found that the islets of Langerhans, where the beta cells reside, had been infiltrated by lymphocytes—a sign of probable autoimmune activity. Soon thereafter Franco Bottazzo of Middlesex Hospital Medical School in London established that blood from patients with type 1 diabetes reacted to islets but that blood of nondiabetics did not, which suggested that autoantibodies targeted to the diabetes’ own beta cells were circulating in the patients’ blood. This finding set off a hunt for the autoantigens—the specific molecular targets of the autoantibodies—in the beta cells, because researchers hoped that discovery of the autoantigens would clarify how diabetes arises.

Intensive research over the past 20 years has uncovered three major pancreatic autoantigens produced in people with newly diagnosed type 1 diabetes: insulin itself, an enzyme called glutamic acid decarboxylase (GAD) and a protein known as islet antigen-2 (IA-2), which was discovered by my group at the National Institutes of Health and is a component of the tiny sacs that ferry insulin around in beta cells. Experts still do not know whether the autoantibodies that bind these proteins play a part in killing beta cells. But they do know, based on highly sensitive detection tests, that one or more are present at diagnosis in some 70 to 90 percent of patients with type 1 diabetes. Today research laboratories use these tests to diagnose type 1 diabetes and distinguish it from type 2 diabetes, which usually arises in overweight adults and does not stem from autoimmunity. (Surprisingly, such tests have uncovered autoantibodies in about 5 percent of patients otherwise diagnosed with type 2 diabetes, which suggests that those individuals have been misclassified or...
LESSONS FROM DIABETES

The idea that autoantibodies (immune system molecules that mistakenly react to the body's own tissues) might serve as early warning signs of later disease came from research into how type 1 diabetes arises. The work revealed that this form of diabetes stems from an autoimmune attack on the beta cells of the pancreas, which are the insulin producers (diagrams), and that autoantibodies targeted to substances made by beta cells appear years before symptoms do (graph). Symptoms arise when too few beta cells are left to meet the body's insulin requirements.

HOW DIABETES DEVELOPS

The attack on beta cells begins when immune cells called T lymphocytes and B lymphocytes invade the islets of Langerhans, where the beta cells reside. The T cells probably cause most of the damage (top detail), but as those cells work their mischief, the B lymphocytes spit out antibodies against proteins made by beta cells, usually starting with insulin.

As the attack on the islets continues, damaging them severely, other types of autoantibodies may appear, such as ones targeted to the proteins GAD and IA-2 (bottom detail). The order and time at which the additional autoantibodies arise can vary.

RISK PROJECTION

Percent Who Will Become Diabetic within Five Years

Number of Autoantibody Types in Blood

AUTOANTIBODIES AND DIABETES RISK

Whether autoantibodies to insulin, GAD and IA-2 contribute to the beta cell killing is not known, but studies have shown that the molecules can signal greatly enhanced risk for diabetes. Risk increases with the number of diabetes-related autoantibody types in the blood.
have a combination of type 1 and type 2 diabetes.)

Interest in the three autoantibodies escalated with the discovery that they appear long before the onset of diabetic symptoms. In studies conducted by various laboratories, investigators took blood samples from thousands of healthy schoolchildren and then monitored the youngsters' health for up to 10 years. When a child came down with type 1 diabetes, the researchers pulled the individual's blood sample out of storage to see whether it contained autoantibodies. The vast majority of children destined to become diabetic had one or more of the three signature diabetes-related autoantibodies in their blood as long as 10 years before any recognizable symptoms arose.

Before this work, some experts thought that type 1 diabetes developed suddenly, perhaps within a matter of weeks. The new data demonstrated, instead, that in most cases the immune system silently assails the pancreas for years until so many beta cells die that the organ can no longer make enough insulin for the body's needs. That is the point when the classic early symptoms of diabetes arise, such as excessive hunger, thirst and urination.

More important, these studies also raised the prospect that doctors might forecast whether a child is at risk for type 1 diabetes by testing blood for the presence of these autoantibodies. Clinical researchers found that an individual with one autoantibody has a 10 percent risk of showing symptoms within five years. With two autoantibodies, the chance of disease jumps to 50 percent; with three autoantibodies, the threat rockets to between 60 and 80 percent.

The ability to predict whether a person is likely to fall ill with type 1 diabetes has had major repercussions for medical researchers trying to better understand and prevent the disease. Before the discovery of predictive autoantibodies, for example, it was almost impossible to conduct clinical trials of new preventive therapies, because the disorder is relatively rare, affecting about one individual in 400. Such odds meant that more than 40,000 subjects would have to be entered into a trial in order to assess the effects of an intervention on the 100 who would eventually be affected.

Now scientists can select for study those people whose blood shows two or more of the diabetes-related autoantibodies, because at least half the subjects, if untreated, will most likely come down with the disease within five years. Slashing the number of subjects who must be enrolled in a prevention trial has made such experiments feasible for the first time. In one investigation, doctors identified several thousand individuals at high risk of diabetes and tested whether injections of insulin could avert the disease. Sadly, this treatment proved unsuccessful; efforts to find useful interventions continue.

The discovery that autoantibodies frequently herald the onset of type 1 diabetes prompted scientists to examine whether the same might be true in other autoimmune diseases. One that has been the focus of especially intense research is rheumatoid arthritis, a debilitating condition that is highly prevalent, affecting about 1 percent of the world's population. In those affected, the immune system attacks and destroys the lining of the joints, causing swelling, chronic pain and eventual loss of movement.

**Predicting Other Diseases**

Autoantibodies have recently unearthed an autoantibody that is present in 30 to 70 percent of patients diagnosed with rheumatoid arthritis. The antibody latches onto citrulline (a modified version of the amino acid arginine), which is present in certain proteins. Studies have now revealed that the autoantibody appears in the bloodstream before the first symptoms turn up, in some cases more than 10 years before. Further, the likelihood that the illness will develop is as much as 15 times greater in people carrying that antibody than in those who lack it.

The knowledge that the anticitrulline autoantibody might serve as a predictive marker is particularly exciting because, in contrast to the situation in type 1 diabetes, doctors already have medicines that might be delivered to prevent or slow the onset of arthritis. Rheumatologists know that quickly and aggressively treating newly diagnosed patients with certain drugs, such as ones that combat inflammation, can retard or sometimes stop the devastating loss of joint flexibility. It is not unreasonable to think therefore that earlier intervention might be even more protective. The hope now is that doctors will be able to screen the general population, or those with a family history of the condition, and then start treating those who make anticitrulline antibodies before autoimmunity irrevocably harms their tissues. First, however, further clinical trials must be carried out to confirm that these autoantibodies accurately predict the onset of joint symptoms. In addition, a reasonably priced test suitable for screening will have to be

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**The Author**

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introduced, along with protocols for deciding exactly who should be tested, when and how often.

For certain other autoimmune disorders, the detection of predictive autoantibodies could potentially enable people to shut down autoimmune activity by avoiding certain triggers in their environment. A case in point is celiac disease. In people with this condition, the gluten protein found in wheat, rye and barley incites the immune system to attack the lining of the small intestine, which then fails to absorb food properly; diarrhea, weight loss and malnourishment then ensue. Patients must eliminate gluten from their diet, bypassing most bread, pasta and cereal for the rest of their lives.

Investigations into the underpinnings of celiac disease have revealed that many patients make an autoantibody that reacts with tissue transglutaminase, an enzyme that modifies many newly made proteins. This autoantibody emerges up to seven years before symptoms do, suggesting that high-risk individuals might forestall the disease entirely by eliminating gluten from their diet. This idea has not yet been tested, however.

More Uses for Autoantibody Tests

Immunologists are exploring whether autoantibodies can serve as early warnings in other ways as well. For instance, some autoantibodies might help doctors to gauge the rate at which an already diagnosed autoimmune condition is likely to progress or how severe it will become.

Patients with multiple sclerosis often start off with relatively mild symptoms that then disappear for a while. Some people continue in remission for a long time or have manageable recurrences. But others grapple with more frequent or severe symptoms, and a few enjoy no remissions at all. Doctors struggle to discern which individuals with early symptoms will go on to suffer from the harshest effects, so that they can counsel the patients accordingly. In 2003 a study of more than 100 individuals with newly identified multiple sclerosis revealed that those who made autoantibodies directed against two proteins that insulate nerve cells were almost four times more likely to suffer a relapse after the initial symptoms abated than were those without the autoantibodies. In addition, the antibody-positive patients relapsed more quickly than the others. These results suggest that testing for these autoantibodies could offer a quick way to predict whether, and how rapidly, multiple sclerosis will advance, although further study is needed before such testing can be put into practice and used to guide therapy.

In the past few years, researchers have made the intriguing finding that autoantibodies can also appear in people with certain disorders not typically thought of as autoimmune conditions, such as some cancers. These autoantibodies probably do not control tumor growth, but laboratories around the world are examining whether they can be useful for the early detection of cancer. In other conditions, such as atherosclerosis, investigators are looking into the possibility that autoantibodies might show which patients are more prone to a blockage in the arteries to the brain and therefore to stroke.

Scientific Challenges

So far much of the work I have discussed has been confined to a small number of academic laboratories and to a few of the major autoimmune diseases. Investigators and companies, however, are now beginning to recognize the potential value of these proteins for improving patient care. They are trying to extend the findings and unearth predictive autoantibodies linked to other autoimmune disorders.

This task is challenging, however, in part because research-

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<th>Disorders under Study</th>
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<td>Investigators have found autoantibodies that might serve as predictors of risk or of progression for a number of autoimmune conditions beyond diabetes, including those listed below.</td>
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<tr>
<th>DISORDER</th>
<th>STATUS OF RESEARCH</th>
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<tr>
<td>Addison's disease (a disorder of the adrenal glands; results in low blood pressure, weakness and weight loss)</td>
<td>Autoantibodies to adrenal tissue and the enzyme 21-hydroxylase are highly predictive in children</td>
</tr>
<tr>
<td>Antiphospholipid syndrome (marked by recurrent clots in blood vessels and pregnancy loss)</td>
<td>Autoantibodies to various molecules appear to signal risk for complications of the disorder</td>
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<tr>
<td>Celiac disease (a digestive disorder triggered by gluten in foods)</td>
<td>Predictive autoantibodies have been identified that target an enzyme called tissue transglutaminase</td>
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<tr>
<td>Multiple sclerosis (neurological condition causing loss of movement)</td>
<td>Autoantibodies to proteins in the myelin sheath that insulates nerve cells appear to predict risk of relapse</td>
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<tr>
<td>Rheumatoid arthritis (chronic joint inflammation)</td>
<td>Autoantibodies to citrulline, a component of many modified proteins, have been found to appear as many as 10 years before symptoms occur</td>
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<tr>
<td>Systemic lupus erythematosus (can affect many organs, including the joints, kidneys and skin)</td>
<td>Several related autoantibodies have been found; one or more of these appear in up to 80 percent of patients before symptoms arise</td>
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ers will have to follow large populations for years to prove that particular autoantibodies can signal future disease. That is, many thousands of healthy people must be recruited to give blood samples and then tracked carefully for 10 years or more to see if they fall sick. Aside from posing logistical difficulties, these prospective studies can cost tens of millions of dollars.

An alternative to conducting prospective studies from scratch might be to tap into existing health databases and carry out retrospective studies. For example, blood samples and medical information have already been collected over many years from members of the U.S. military and from subjects in the Women’s Health Initiative, a vast, ongoing study of more than 100,000 women. Experts in autoimmunity could team up with investigators in these and other projects, identify individuals who have been diagnosed with an autoimmune disease and then examine their stored blood for the presence of predictive autoantibodies. This approach would be relatively inexpensive and could yield rapid results—and a few researchers have already embarked on such collaborations.

A second avenue of attack would involve identifying heretofore unrecognized autoantigens. One could search human genetic databases for the sequences that encode proteins and use this information to manufacture these proteins in the lab.

Scientists could pinpoint those that are autoantigens by mixing each of the manufactured proteins with blood from patients who have an autoimmune disease and allowing complexes of proteins and antibodies to form. Analyses of such complexes could identify both the autoantigens in the collection and the autoantibodies that recognize them. That done, the predictive value of the autoantibodies could be determined in a prospective or retrospective study.

This full-genome approach to isolating autoantigens is difficult. Nevertheless, a handful of research groups are now screening smaller batches of proteins in this way. In my laboratory, for example, we are hunting for new autoantigens involved in type 1 diabetes by manufacturing dozens of selected pancreatic proteins known to be involved in the secretion of insulin and testing whether autoantibodies in blood from diabetics bind these proteins.

**Practical Challenges**

*Medicine as we know it is evolving from diagnosing and treating diseases after they develop to predicting and preventing them. Ten or 20 years from now autoantibody screening for at least some diseases will almost certainly become a familiar part of the standard medical examination.*

**CHECKUPS OF THE FUTURE**

Someday physicals could routinely include screening for autoantibodies.

1. A blood sample would be given to a laboratory, which would extract the plasma (the acellular part).

2. The plasma would be washed over a chip containing an array of autoantigens—molecules known to be capable of eliciting autoimmune reactions—at known positions. Any autoantibodies in the blood would bind to the autoantigens, triggering signals indicating the identity and quantity of the bound autoantibodies.

3. This information would be translated into a prediction of the patient’s risk for becoming afflicted with specific conditions.
In the future, patients visiting their doctors for a physical might have their blood tested for multiple predictive autoantibodies in a single test. In one plausible scenario, the doctor would send a blood sample to a lab for an autoantibody analysis, along with standard tests for cholesterol, blood glucose and other health indicators. There a machine would pass the blood over a tiny chip displaying an array of known autoantigens. Autoantibodies in the blood that bound to one or more of these antigens would trigger pulses of light that would be picked up by a detector. Within hours, the doctor would receive a readout translating this information into a health forecast.

The presence of predictive autoantibodies would not mean that a patient will definitely get sick, but would give a percentage risk of diabetes and numerous other conditions developing over some number of months or years.

These tests might even be combined with other biological assays to give more accurate health predictions. In the case of type 1 diabetes, possession of certain forms of genes that regulate the immune system, called HLA genes, are also known to correlate with disease risk. A prognostic assay might combine tests for those HLA variants with tests for predictive autoantibodies.

The vision of prediction is an enticing one, but even after the challenges of identifying predictive autoantibodies have been overcome, other issues will need to be resolved in preparation for their use in the clinic. One critical question relates to cost. At present, lab screening for predictive autoantibodies is cumbersome and labor-intensive. Widespread population screening for multiple autoantibodies will become practical only when rapid, inexpensive automated methods for detecting them are designed. To date, only a few small biotechnology companies are trying to devise such methods.

Another issue to be decided is which people should be screened and how often. It is unreasonable to test children for diseases that occur only in adults, and the converse would also hold true. Similarly, the frequency of screening would have to depend in part on whether autoantibodies tend to arise many years or just a few months before the onset of clinical symptoms.

**Problems of Prediction**

Before autoantibodies are widely used to foretell a patient’s risk of future disease, many tough ethical and practical issues must be considered.

- Should doctors test for diseases that have no preventive treatment or cure?
- What is the best way to make sure patients understand that a positive test does not mean disease will definitely develop but indicates a given probability of risk?
- How can the risks of false positive or negative tests be minimized so that few patients are unnecessarily alarmed or mistakenly reassured?
- Is the cost of routine screening justified by the number of patients who would be found to be at risk and able to benefit from early treatment?
- For autoimmune diseases that run in families, should family members of afflicted individuals be tested, and will the worry over a result indicating high risk be easier to live with than the anxiety of not knowing?
- Will a positive test lead to discrimination from employers, health insurers or society in general?

By far the most important factor controlling whether predictive autoantibody screening will become widespread is the availability of therapy. Some would argue that embarking on predictive testing for diseases makes little sense if patients can be offered no preventive or ameliorating treatment. A large and intensive research effort is under way to develop new therapies for autoimmune diseases, but because the conditions are so complicated and varied, progress may not come quickly.

Of course, the ability to forecast someone’s life and death raises thorny ethical issues. Some people may choose not to know that they are likely to come down with a given disease, and doctors must be careful to respect that decision. Patients may also be concerned that insurers or employers could obtain medical information and use it to discriminate against them, even while they are healthy. As is true of genetic testing, such issues call for in-depth discussion.

Forecasts of the future have always intrigued and frightened people. Handled properly, though, such knowledge could benefit the millions of patients and doctors destined to battle autoimmune diseases. By making early intervention possible, predictive autoantibodies have the potential to alleviate much misery and to help provide extra years of healthy life.

**MORE TO EXPLORE**