My vote for the most important scientific revolution of all time would trace back 10,000 years ago to the Middle East, when people first noticed that new plants arise from seeds falling to the ground from other plants—a realization that led to the birth of agriculture. Before that observation, the human race had based its diet on fruits, nuts, tubers and occasional meats. People had to move to where their food happened to be, putting them at the mercy of events and making long-term settlements impossible.

Once humans uncovered the secret of seeds, they quickly learned to domesticate crops, ultimately crossbreeding different grass plants to create such staple grains as wheat, rye and barley, which were nutritious, versatile, storable, and valuable for trade. For the first time, people were able to abandon the nomadic life and build cities. It is no coincidence that the first agricultural areas also became “cradles of civilization.”

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The advancement, however, came at a dear price: the emergence of an illness now known as celiac disease (CD), which is triggered by ingesting a protein in wheat called gluten or eating similar proteins in rye and barley. Gluten and its relatives had previously been absent from the human diet. But once grains began fueling the growth of stable communities, the proteins undoubtedly began killing people (often children) whose bodies reacted abnormally to them. Eating such proteins repeatedly would have eventually rendered sensitive individuals unable to properly absorb nutrients from food. Victims would also have come to suffer from recurrent abdominal pain and diarrhea and to display the emaciated bodies and swollen bellies of starving people. Impaired nutrition and a spectrum of other complications would have made their lives relatively short and miserable.

If these deaths were noticed at the time, the cause would have been a mystery. Over the past 20 years, however, scientists have pieced together a detailed understanding of CD. They now know that it is an autoimmune disorder, in which the immune system attacks the body’s own tissues. And they know that the disease arises not only from exposure to gluten and its relatives had previously been absent from the human diet. But once grains began fueling the growth of stable communities, the proteins undoubtedly began killing people (often children) whose bodies reacted abnormally to them. Eating such proteins repeatedly would have eventually rendered sensitive individuals unable to properly absorb nutrients from food. Victims would also have come to suffer from recurrent abdominal pain and diarrhea and to display the emaciated bodies and swollen bellies of starving people. Impaired nutrition and a spectrum of other complications would have made their lives relatively short and miserable.

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What is more, CD provides an illuminating example of the way such a triad—an environmental trigger, susceptibility genes and a gut abnormality—may play a role in many autoimmune disorders. Research into CD has thus sug-
gested new types of treatment not only for the disease itself but also for various other autoimmune conditions, such as type 1 diabetes, multiple sclerosis and rheumatoid arthritis.

**Early Insights**

After the advent of agriculture, thousands of years passed before instances of seemingly well-fed but undernourished children were documented. CD acquired a name in the first century A.D., when Aretaeus of Cappadocia, a Greek physician, reported the first scientific description, calling it *koiliakos*, after the Greek word for “abdomen,” *koelia*. British physician Samuel Gee is credited as the modern father of CD. In a 1887 lecture he described it as “a kind of chronic indigestion which is met with in persons of all ages, yet is especially apt to affect children between one and five years old.” He even correctly surmised that “errors in diet may perhaps be a cause.” As clever as Gee obviously was, the true nature of the disease escaped even him, as was clear from his dietary prescription: he suggested feeding these children thinly sliced bread, toasted on both sides.

Identification of gluten as the trigger occurred after World War II, when Dutch pediatrician Willem-Karel Dicke noticed that a war-related shortage of bread in the Netherlands led to a significant drop in the death rate among children affected by CD—from greater than 35 percent to essentially zero. He also reported that once wheat was again available after the conflict, the mortality rate soared to previous levels. Following up on Dicke’s observation, other scientists looked at the different components of wheat, discovering that the major protein in that grain, gluten, was the culprit.

Turning to the biological effects of gluten, investigators learned that repeated exposure in foods containing wheat, rye or barley trigger an autoimmune reaction (against the body’s own tissues) in people afflicted with celiac disease. The response harms the intestinal lining and impairs the body’s absorption of nutrients. Chronic exposure to those foods can also lead to cancer and other ill effects in such individuals.
CD patients causes the villi, fingerlike structures in the small intestine, to become chronically inflamed and damaged, so that they are unable to carry out their normal function of breaking food down and shunting nutrients across the intestinal wall to the bloodstream (for delivery throughout the body). Fortunately, if the disease is diagnosed early enough and patients stay on a gluten-free diet, the architecture of the small intestine almost always returns to normal, or close to it, and gastrointestinal symptoms disappear.

In a susceptible person, gluten causes this inflammation and intestinal damage by eliciting activity by various cells of the immune system. These cells in turn harm healthy tissue in an attempt to destroy what they perceive to be an infectious agent.

A Diagnostic Discovery
Fuller details of the many mechanisms through which gluten affects immune activity are still being studied, but one insight in particular has already proved useful in the clinic: a hallmark of the aberrant immune response to gluten is production of antibody molecules targeted to an enzyme called tissue transglutaminase. This enzyme leaks out of damaged cells in inflamed areas of the small intestine and attempts to help heal the surrounding tissue.

Discovery that these antibodies are so common in CD added a new tool for diagnosing the disorder and also allowed my team and other researchers to assess the incidence of the disease in a new way—by screening people for the presence of this antibody in their blood. Before then, doctors had only nonspecific tests, and thus the most reliable way to diagnose the disease was to review the patient’s symptoms, confirm the intestinal inflammation by taking a biopsy of the gut, and assess whether a gluten-free diet relieved symptoms. (Screening for antibodies against gluten is not decisive, because they can also occur in people who do not have CD.)

For years CD was considered a rare disease outside of Europe. In North America, for example, classic symptoms were recognized in fewer than one in 10,000 people. In 2003 we published the results of our study—the largest hunt for people with CD ever conducted in North America, involving more than 13,000 people. Astoundingly, we found that one in 133 apparently healthy subjects was affected, meaning the disease was nearly 100 times more common than had been thought. Work by other researchers has confirmed similar levels in many countries, with no continent spared.

How did 99 percent of cases escape detection for so long? The classical outward signs—persistent indigestion and chronic diarrhea—appear only when large and crucial sections of the intestine are damaged. If a small segment of the intestine is dysfunctional or if inflammation is fairly mild, symptoms may be less dramatic or atypical.

It is also now clear that CD often manifests in a previously unappreciated spectrum of symptoms driven by local disruptions of nutrient absorption from the intestine. Disruption of iron absorption, for example, can cause anemia, and poor folate uptake can lead to a variety of neurological problems. By robbing the body of particular nutrients, CD can thus produce such symptoms as osteoporosis, joint pain, chronic fatigue, short stature, skin lesions, epilepsy, dementia, schizophrenia and seizure.

Because CD often presents in an atypical fashion, many cases still go undiagnosed. This new ability to recognize the disease in all its
forms at an early stage allows gluten to be removed from the diet before more serious complications develop.

### From Gluten to Immune Dysfunction

Celiac disease provides an enormously valuable model for understanding autoimmune disorders because it is the only example where the addition or removal of a simple environmental component, gluten, can turn the disease process on and off. (Although environmental factors are suspected of playing a role in other autoimmune diseases, none has been positively identified.)

To see how gluten can have a devastating effect in some people, consider how the body responds to it in most of the population. In those without CD, the body does not react. The normal immune system jumps into action only when it detects significant amounts of foreign proteins in the body, reacting aggressively because the foreigners may signal the arrival of disease-causing microorganisms, such as bacteria or viruses.

A major way we encounter foreign proteins and other substances is through eating, and immune soldiers sit under the epithelial cells that line the intestine (enterocytes), ready to pounce and call in reinforcements. One reason our immune system typically is not incited by this thrice-daily protein invasion is that before our defenses encounter anything that might trouble them, our gastrointestinal system usually breaks down most ingested proteins into standard amino acids—the building blocks from which all proteins are constructed.

Gluten, however, has a peculiar structure: it is unusually rich in the amino acids glutamine and proline. This property renders part of the molecule impervious to our protein-chopping machinery, leaving small protein fragments, or peptides, intact. Even so, in healthy people, most of these peptides are kept within the gastrointestinal tract and are simply excreted before the immune system even notices them. And any gluten that sneaks across the gastrointestinal lining is usually too minimal to excite a significant response from a normally functioning immune system.

CD patients, on the other hand, have inherited a mix of genes that contribute to a heightened immune sensitivity to gluten. For example, certain gene variants encoding proteins known as histocompatibility leukocyte antigens (HLAs) play a role. Ninety-five percent of people with CD possess the gene either for HLA-DQ2 or for HLA-DQ8, whereas just 30 to 40 percent of the general population have one of those versions. This finding and others suggest that the HLA-DQ2 and HLA-DQ8 genes are not the sole cause of immune hyperactivity but that the disease, nonetheless, is nearly impossible to establish without one of them. The reason these genes are key becomes obvious from studies of the function of the proteins they specify.

The HLA-DQ2 and HLA-DQ8 proteins are made by antigen-presenting cells. These immune sentinels gobble up foreign organisms and proteins, chop them, fit selected protein fragments into grooves on HLA molecules, and display the resulting complexes on the cell surface for perusal by immune system cells called helper T lymphocytes. T cells that can recognize and bind to the displayed complexes then call in reinforcements.

In patients with CD, tissue transglutaminase released by intestinal epithelial cells attaches to undigested gluten and modifies the peptides in a way that enables them to bind extremely strongly to DQ2 and DQ8 proteins. In conse-

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**FAST FACTS**

- Roughly 1 percent of the global population has celiac disease, although most do not know it.
- More than two million people in the U.S. are afflicted with the disease.
- Some common symptoms in infants and children are abdominal pain, bloating, constipation, diarrhea, weight loss and vomiting.
- About half of adults with the condition do not suffer from diarrhea at diagnosis.
- Other signs that may occur in adults are anemia, arthritis, bone loss, depression, fatigue, infertility, joint pain, seizures, and numbness in the hands and feet.

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**[THE AUTHOR]**

Alessio Fasano is professor of pediatrics, medicine and physiology and director of the Mucosal Biology Research Center and the Center for Celiac Research at the University of Maryland School of Medicine. Much of his basic and clinical research focuses on the role of intestinal permeability in the development of celiac disease and other autoimmune disorders.
Three factors underlie celiac disease: an environmental trigger, a genetic susceptibility and, according to the author’s research, an unusually permeable gut (below). The author suspects that the same basic triad contributes to other autoimmune diseases, although each disorder will have its own triggers and genetic components.

**A Trio of Causes**

**Genetic Predisposition**

Almost all patients harbor a gene for either the HLA-DQ2 protein or the HLA-DQ8 protein, or both. These HLA molecules display gluten fragments to immune system cells, which then direct an attack on the intestinal lining. Other genes are likely to be involved as well, but these additional culprits may differ from person to person.

**Leaky Small Intestine**

In most people, links known as tight junctions “glue” intestinal cells together. In those with celiac disease, the junctions come apart, allowing a large amount of indigestible gluten fragments to seep into the underlying tissue and incite immune system cells. Treatments that reduced leakiness could potentially ease not only celiac disease but also other autoimmune disorders involving unusually permeable intestines.

**Finding the Leak**

It is fair to say that the theory that a leaky gut contributes to CD and autoimmunity in general was initially greeted with great skepticism, partly because of the way scientists thought of the intestines. When I was a medical student in the 1970s, the small intestine was described as a pipe composed of a single layer of cells connected like tiles with an impermeable “grout,” known as tight junctions, between them. This simple model of the tight junctions as inert, impermeable filler did not inspire legions of researchers to study their structure, and I was among the unenthused.

It was only an unexpected twist of fate, and one of the most disappointing moments of my career, that drew me to study tight junctions. In the late 1980s I was working on a vaccine for...
cholera. At that time, the cholera toxin was believed to be the sole cause of the devastating diarrhea characteristic of that infection. To test this hypothesis, my team deleted the gene encoding the cholera toxin from the bacterium *Vibrio cholerae*. Conventional wisdom suggested that bacteria disarmed in this way would make an ideal vaccine, because the remaining proteins on a living bacterial cell would elicit a strong immune response that would protect against diarrhea.

But when we administered our attenuated bacteria to volunteers, the vaccine provoked enough diarrhea to bar its use. I felt completely disheartened. Years of hard work were literally down the toilet, and we were faced with two unattractive options: giving up and moving on to another research project or persevering and trying to understand what went wrong. Some intuition that there was more to this story prompted us to choose the latter path, and this decision led us to discover a new toxin that caused diarrhea by a previously undescribed mechanism. It changed the permeability of the small intestine by disassembling those supposedly inert tight junctions, an effect that allowed fluid to seep from tissues into the gut. This “grout” was interesting after all.

Indeed, at nearly the same time, a series of seminal discoveries clarified that a sophisticated meshwork of proteins forms the tight junctions; however, little information was available on how these structures were controlled. Therefore, the discovery of our toxin, which we called the “zonula occludens toxin,” or Zot (zonula occludens is Latin for “tight junction”), provided a valuable tool for clarifying the control process. It revealed that a single molecule, Zot, could loosen the complex structure of the tight junctions. We also realized that the control system that made this loosening possible was too complicated to have evolved simply to cause biological harm to the host. *V. cholerae* must cause diarrhea by exploiting a preexisting host pathway that regulates intestinal permeability.

Five years after the formulation of this hypothesis, we discovered zonulin, the protein that in humans and other higher animals increases intestinal permeability by the same mechanism as the bacterial Zot. How the body uses zonulin to its advantage remains to be established. Most likely, though, this molecule, which is secreted by intestinal epithelial tissue as well as by cells in other organs (tight junctions have important roles in tissues throughout the body), performs several jobs—including regulating the movement of fluid, large molecules and immune cells between body compartments.

Discovery of zonulin prompted us to search the medical literature for human disorders characterized by increased intestinal permeability. It was then that we first learned, much to my surprise, that many autoimmune diseases—among them, CD, type 1 diabetes, multiple sclerosis, rheumatoid arthritis and inflammatory bowel diseases—all have as a common denominator aberrant intestinal permeability. In many of these diseases, the increased permeability is caused by abnormally high levels of zonulin. And in CD, it is now clear that gluten itself prompts exaggerated zonulin secretion (perhaps because of the patient’s genetic makeup).

This discovery led us to propose that it is the enhanced intestinal permeability in CD patients that allows gluten, the environmental factor, to seep out of the gut and to interact freely with genetically sensitized elements of the immune system. That understanding, in turn, suggests that removing any one factor of the autoimmunity-causing trinity—the environmental trigger, the heightened immune reactivity or the intestinal permeability—should be enough to stop the disease process.

**Therapies to Topple the Trinity**

As I mentioned before, and as this theory would predict, removing gluten from the diet ends up healing the intestinal damage. Regrettably, a lifelong adherence to a strict gluten-free diet is not easy. Gluten is a common and, in many countries, unlabeled ingredient in the human diet. Further complicating adherence, gluten-free products are not widely available and are more

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**A Clue to Delayed Onset**

People with celiac disease are born with a genetic susceptibility to it. So why do some individuals show no evidence of the disorder until late in life? In the past, I would have said that the disease process was probably occurring in early life, just too mildly to cause symptoms. But now it seems that a different answer, having to do with the bacteria that live in the digestive tract, may be more apt.

These microbes, collectively known as the microbiome, may differ from person to person and from one population to another, even varying in the same individual as life progresses. Apparently they can also influence which genes in their hosts are active at any given time. Hence, a person whose immune system has managed to tolerate gluten for many years might suddenly lose tolerance if the microbiome changes in a way that causes formerly quiet susceptibility genes to become active. If this idea is correct, celiac disease might one day be prevented or treated by ingestion of selected helpful microbes, or “probiotics.” —A.F.
The theory that a leaky gut contributes to CD and autoimmunity in general was initially greeted with skepticism.

Investigators do not know every detail of how the immune system wreaks havoc with the intestinal lining of celiac patients, but they have identified a number of likely processes (below). Colored arrows indicate events that might be blocked by interventions now being investigated [see table on opposite page].

1. Indigestible fragments of gluten induce enterocytes to release the protein zonulin, which loosens tight junctions.

2. Gluten fragments cross the intestinal lining in abundance and accumulate under epithelial cells (enterocytes).

3. The gluten induces enterocytes to secrete interleukin-15 (IL-15), which arouses immune cells called intraepithelial lymphocytes against enterocytes.

4. Tissue transglutaminase (TTG), an enzyme released by the damaged cells, modifies the gluten.

5. Antigen-presenting cells of the immune system join the modified gluten to HLA molecules and display the resulting complexes to other immune cells: helper T cells.

6. Helper T cells recognize the complexes secrete molecules that attract other immune cells and can directly damage enterocytes.

7. Helper T cells spur killer T cells to directly attack enterocytes.

Consequently, several alternative therapeutic strategies have been considered that disrupt at least one element of the three-step process. Alvine Pharmaceuticals in San Carlos, Calif., has developed oral protein-enzyme therapies that completely break down gluten peptides normally resistant to digestion and has an agent in clinical trials. Other investigators are considering ways to inhibit tissue transglutaminase so that it does not chemically modify undigested gluten fragments into the form where they bind effectively to HLA-DQ2 and HLA-DQ8 proteins.

No one has yet come up with safe and ethical ways to manipulate the genes that make people susceptible to disease. But researchers are busy developing therapies that might dampen some of the genetically controlled factors that contribute to the immune system’s oversensitivity. For example, the Australian company Nexep is working on a vaccine that would expose the immune system to small amounts of strongly immunogenic forms of gluten, on the theory that repeated small exposures would ultimately induce the immune system to tolerate gluten.

With an eye toward blocking the intestinal...
Today patients with celiac disease have one therapeutic option: avoid all foods that contain gluten. But because following a restricted diet can be difficult, investigators are exploring other options for patients, such as those listed below. These are early days in the process; no drug in the table has yet reached the advanced clinical trials needed to gain marketing approval.

<table>
<thead>
<tr>
<th>THERAPY</th>
<th>DRUG NAME (INVESTIGATOR/STATUS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avoid gluten in the diet of infants through their first year of life</td>
<td>No drug (University of Maryland and, separately, Marche Polytechnic University, Italy/in human trials)</td>
</tr>
<tr>
<td>Degraded otherwise indigestible gluten fragments so they cannot evoke an immune response</td>
<td>ALV003 (Alvine and, separately, AN-PEP at VU University Medical Center, the Netherlands/in human trials)</td>
</tr>
<tr>
<td>Block zonulin from making the gut permeable</td>
<td>Larazotide (Alba Therapeutics/in human trials)</td>
</tr>
<tr>
<td>Keep tissue transglutaminase from modifying gluten fragments in ways that stimulate the immune system</td>
<td>No name (Numerator and Stanford University/under study in the laboratory)</td>
</tr>
<tr>
<td>Stop HLA-DQ2 from attaching to gluten peptides and displaying them to helper T cells</td>
<td>Mimics of gluten (Leiden University, the Netherlands, and, separately, Stanford University/under study in the laboratory)</td>
</tr>
<tr>
<td>Vaccinate patients with selected gluten fragments to induce helper T cells to tolerate, rather than reacting to, gluten displayed by HLA-DQ2 molecules</td>
<td>Nexvax2 (Nexpep, Australia/in human trials)</td>
</tr>
<tr>
<td>Block migration of killer T cells into the intestinal lining</td>
<td>CCX282-B (Chemocentryx/in human trials)</td>
</tr>
<tr>
<td>Start a hookworm infection (the parasites dampen a host’s immune responses in the gut)</td>
<td>Hookworm parasites (Princess Alexandra Hospital, Australia, and collaborators/in human trials)</td>
</tr>
</tbody>
</table>

Gluten and TTG. Those antibodies might cause further damage when they hit their targets on or near enterocytes, but the role of antibodies in the disease is unclear.

Together the tests showed no excess of side effects in patients given Larazotide rather than the placebo. More important, the first, smaller study demonstrated that the agent reduced gluten-induced intestinal barrier dysfunction, production of inflammatory molecules and gastrointestinal symptoms in celiac patients. And the second, large study, reported at a conference in April, showed that CD patients who received a placebo produced antibodies against tissue transglutaminase but that the treated group did not. As far as I know, this result marks the first time a drug has halted an autoimmune process, interfering specifically with an immune response against a particular molecule made by the body. Other drugs that suppress immune activity act less specifically. Recently Alba received approval from the U.S. Food and Drug Administration to expand studies of Larazotide to other autoimmune disorders, including type 1 diabetes and Crohn’s disease.

These new prospects for therapy do not mean that CD patients can abandon dietary restrictions anytime soon. Diet could also be used in a new way. Under the leadership of Carlo Catassi, my team at the University of Maryland has begun a long-term clinical study to test whether having infants at high risk eat nothing containing gluten until after their first year can delay the onset of CD or, better yet, prevent it entirely. “High risk,” in this case, means infants possess susceptibility genes and their immediate family has a history of the disorder.

We suspect the approach could work because the immune system matures dramatically in the first 12 months of life and because research on susceptible infants has implied that avoiding gluten during the first year of life might essentially train that developing immune system to tolerate gluten thereafter, as healthy people do, rather than being overstimulated by it. So far we have enrolled more than 700 potentially genetically susceptible infants in this study, and preliminary findings suggest that delaying gluten exposure reduces by fourfold the likelihood that CD will develop. It will be decades, however, until we know for certain whether this strategy can stop the disease from ever occurring.

Given the apparently shared underpinning of autoimmune disorders in general, researchers who investigate those conditions are eager to learn whether some therapeutic strategies for CD might also ease other autoimmune conditions that currently lack good treatments. And with several different approaches in the pipeline to treat CD, we can begin to hope that this disease, which has followed humanity from the dawn of civilization, is facing its last century on earth.