reaches full fighting strength after passing through the lymphoid tissue scattered strategically throughout the body. With every breath we take, untold numbers of T cells and B cells silently and efficiently eliminate viruses and bacteria that have breached the outer ramparts of the body's fortress. Until very recently—effective antibiotics first appeared less than a century ago—our immune system was all we had to protect us against the world of microbes.

Not surprisingly, a critical failure in one of the genes that code for the proteins necessary to have a well-organized army of T cells and navy of B cells poses a supreme threat to survival. A failed immune system is of as little defensive value to us as were the undisciplined mercenaries in the depleted legions of 5th-century Rome before the advancing cavalry from the steppes of Asia. Death comes quickly if your immune system is badly flawed. It comes more slowly if it is only weakened. Indeed, one of the theories offered to explain the natural limits on life span is the declining strength of our immune defenses after six or seven decades. There is a good reason why physicians used to call pneumonia "the old man's friend." Infections that we usually can fight off in midlife often take elderly people despite antibiotic therapy.

There are several dozen inherited immunodeficiency disorders, each caused by the failure of one of many different genes, most of which code for proteins involved in making T cells. All are rare—so rare that, once her training at a big medical center is complete, the average pediatrician never again sees a single patient with one of them. Nevertheless, these diseases are exceedingly important, because as we try help the children afflicted with them, we learn a great deal about the nature of the immune system. They are also extremely interesting for another reason: Genetically determined immune disorders are among the leading candidates for effective use of gene therapy. In particular, prospects for the future of gene therapy will be significantly influenced by the struggle to cure a disorder called X-linked severe combined immune deficiency (SCID).

X-linked SCID is the most common of the uniformly rare genetic immune disorders, affecting about 1 in every 25,000 boys. It affects only boys because the underlying defect is a mutation in a gene on the long arm of the X chromosome, and the Y chromosome does not carry a normal version of the gene to compensate. Fortunately, almost all girls with a mutation on one X chromosome have a normal counterpart, which is enough to provide them with a healthy defense system. However, in each of her pregnancies, a woman who carries a copy of the mutated gene on one of her Xs has a one-in-four risk of having an affected son (a one-in-two chance of having a son multiplied by the one-in-two chance that he inherited the X with the mutation).

When a family history for the disorder is known, it is possible to offer a genetic test to a woman who is at risk to see if she inherited the SCID mutation. Women who learn they are carriers and who wish to avoid the birth of an affected son can choose the option of prenatal diagnosis and selective abortion of affected fetuses. It is also possible to offer a technique called preimplantation genetic testing that would permit them to bear only girls, thus avoiding the tragic choice of aborting a wanted pregnancy. Given the small size of families during the last 50 years, the rarity of the disorder, and the fact that, until recently, affected babies often died quickly and without a correct diagnosis, many women who are at risk for bearing affected sons may have no hint of that fact. In addition, an unknown number of cases are caused by new mutations.

The causative mutations are found in a gene called the Interleukin-2 receptor gamma chain gene (IL2RGC) that codes for a protein which is part of a molecule that is absolutely necessary for the maturation of active T and B cells. The molecule is a receptor that sits on the surface of the developing immune cells and permits them to take up other chemicals which act as growth factors. Infants born with this X-linked SCID have few T cells and produce no antibodies. Although experts have shown that, depending on the particular mutation, some cases of SCID are more severe than others, almost all affected boys are ill nearly from birth with recurring infections.

These are not merely the normal viral illnesses of childhood. Children with SCID are at high risk for bacterial pneumonia, meningitis, and sepsis. They regularly become infected with organisms that fail to disturb children with normal immunity. Among the most severe is pneumonia caused by an organism called *Pneumocystis carinii*, the same bug that has killed so many patients with AIDS. Infection with chickenpox is potentially fatal. Because the children lack the defenses to contain the varicella virus, it can spread throughout their lungs and even the brain. Risk from

other common viruses such as those that cause the flu, cold sores, and diarrhea is also very high. In fact, many children with SCID suffer from chronic diarrhea because so many foreign organisms have taken up residence in their intestines. The children should not be given live vaccines such as for chickenpox; in them such vaccines can cause the very diseases they are designed to prevent.

Untreated, most children with X-linked SCID would not survive beyond age two. Fortunately, SCID is a disease involving cells that arise in the bone marrow. Thus, when a perfectly matched donor is available, a bone marrow transplant cures the affected child in about 80–90% of cases. Unfortunately, because of the complex nature of our immune defense system, only about 20% of patients have relatives so closely matched that a bone marrow transplant is feasible. Although the National Marrow Donor Program has more than 4.5 million names of potential donors, the odds of finding a match among strangers through its registry are relatively low. The immune system is so genetically complex that it is rare to find matches among non-relatives. Thus, for the 80% of patients without a donor, the best hope for cure is gene therapy—the molecular transplantation of a healthy version of the SCID gene.

Despite its rarity, X-linked SCID is relatively familiar to the public, largely because of a dramatic attempt more than 20 years ago to raise an affected child in a sterile environment for as long as possible. The famous "Bubble Boy" who lived near Houston (and about whom a television movie was later made) spent a decade in a clear plastic sterile chamber, while his parents waited and prayed for a cure. His life ended when he decided he wanted to take the chance of cure by accepting a bone marrow transplant from his sister. The heroic effort failed; he died from a massive viral infection that probably traveled with the donated bone marrow into his body. The overall success rate with such operations was considerably less back then than it is today.

Although every nucleated cell contains the IL2RGC gene, X-linked SCID can be thought of as a disease of the bone marrow, the tissue which contains the stem cells that produce the billions of new recruits that the armed forces of immunity unendingly demand. This is why the disease has long been of interest to gene therapists. It is relatively easy to remove bone marrow cells from patients and to isolate the stem cells. In addition, it has

become possible to isolate and transfer normal IL2RGC DNA into those cells and then infuse them (by a simple intravenous line) into the patient. Miraculously, some of the cells that return by IV find their way back home to the bone marrow. If enough genetically engineered stem cells resume their duties, as they repeatedly divide (which is exactly what stem cells are programmed to do), they create a new army of healthy immune cells in which there is a normal copy of the key gene.

But how can genes, tiny bits of DNA, be taken up by bone marrow cells in a plastic flask and incorporated into a functional spot in their nuclei? The answer lies in genetic engineering. For somatic cell gene therapy to work, genetic engineers must develop a method to attach a normal copy of the gene of interest to a virus (called a vector) that has the capacity to penetrate human cells, enter their nuclei, and insert into their DNA. Scientists have been trying to develop highly effective viral vectors for years, with mixed results. Ironically, in attempting to cure SCID, they used a virus to invade the same cells that they wished to render competent so that the patient would make T and B cells to prevent such viral invasions!

The first impressive success in human gene therapy in SCID occurred at the Necker Hospital for Sick Children in Paris during 1999–2000, a time when the field was at its nadir. Less than a year earlier, a young man named Jesse Gelsinger, who was burdened with a relatively mild form of a different genetic disorder, had died of liver failure after agreeing to undergo gene therapy as part of a research project at the University of Pennsylvania. Subsequent investigation of the case led to censure of the head researcher on several grounds, not the least of which was that he had an ownership interest in a gene therapy company. This tragic occurrence reverberated throughout the research community; for a short time, the National Institutes of Health suspended all human gene therapy research conducted with federal funds in the United States.

The team at the Necker Hospital is led by Alain Fischer, who began his research on gene therapy for X-linked SCID in 1993, just after the culprit gene was identified. He spent six years studying the therapy in mice with a similar form of the disease before trying to treat children. In April of 2000, Dr. Fischer reported that his team had performed gene therapy on two infant boys. Three months later, the little patients were able to leave their protected hospital environments for home. Seven months after that, blood

tests showed that these boys had adequate numbers of normally functioning T and B cells. During 2000, Fischer's team treated eight more young boys with the disorder. After just a few months, all but one had made sufficient progress to enable them to leave the hospital's protective cocoon. Similar therapeutic research efforts were quickly initiated in the United States and several other countries. The impact on the understandably desperate patient community was profound. Parents of affected children who lacked matched donors clamored for gene therapy. Gene therapy researchers were euphoric; the triumph in France was a new beginning. But the euphoria was short-lived.

In the autumn of 2002, the team at Necker Hospital discovered that one of the nine children who had apparently been cured of X-linked SCID with gene therapy had developed a form of T-cell leukemia called T-cell acute lymphoblastic leukemia (ALL) about 30 months after treatment. In essence, the T cells in this child underwent a population explosion. Fortunately, the little boy responded to standard chemotherapy for T-cell leukemia, but the event cast a pall over both the research and the patient communities. The same question now haunted parents and physicians alike. Would more of the treated children develop T-cell leukemia? Because two relatives of the child with leukemia had also developed cancer at an unusually early age, many held out hope that this was an isolated incident, a fluke. But T-cell leukemia is sufficiently rare that a second case would unquestionably point to the gene therapy as the cause.

The second case of T-cell ALL was diagnosed four months after the first. In the United States, the FDA immediately stopped 27 gene therapy trials involving several hundred patients, all of whom were to be treated with retrovirus vectors similar to the one used at Necker Hospital to deliver the genes to the bone marrow cells. Similar actions were taken throughout Europe. Other gene therapy trials that did not involve manipulation of stem cells were allowed to continue because they were viewed as less risky.

What caused leukemia in these children? When researchers use viruses to carry new genes into bone marrow cells, they cannot dictate where in the cell's genome the virus will insert itself. For reasons that are not fully understood, some stretches of DNA are more receptive to insertion than are others. Careful study of the DNA in the leukemia cells of the two boys

revealed that in both cases the viral vector integrated close to a gene called LMO2, a known oncogene (one that influences cell growth and division and which helps prevent cancer). LMO2, which is located on the short arm of chromosome 11, codes for a protein that plays its most important role early in the development of the cells that eventually become immune cells, red blood cells, cells that line the vessels, and even nerve cells. Studies in mice have shown that if LMO2 does not shut down (stop working) when it is supposed to, the mice develop T-cell leukemia.

For statistical reasons, there are grounds to suspect that in a few of the millions of cells used in the gene therapy for X-linked SCID, the virus inserted in or near the promoter region of LMO2 in a way that reactivated it. Each child received about 90 million cells that had been infected with the virus carrying the normal IL2RG gene. Assuming that there are about 30,000 genes in the human genome and that the virus inserts randomly, it is certainly plausible that among the millions of insertion events, at least 100 resulted in cells in which LMO2 was reactivated. Each of those cells can be thought of as posing a risk for T-cell leukemia. Such cells constitute a double whammy to the child's bone marrow. The successful placement of IL2RG caused T-cell proliferation; the inadvertent reactivation of LMO2 blocked their development. This left a growing reservoir of immature T cells. Such cells are vulnerable to other mutations that, as they accumulate, can result in their rapid expansion in number. This is the essence of leukemia—a stampede of white cells.

During 2002–2003, Dr. Fischer's team at Necker worked intensively to modify the gene therapy protocols to reduce the risk. There was good news early in 2004 when studies of leukemias induced in mice by gene therapy with retroviruses like the one used in humans suggested that the problems in SCID might be unique to that disease. This meant that other types of gene therapy might not carry the risk. In May, the French health agency charged with oversight of gene therapy authorized the resumption of gene therapy for X-linked SCID. The new work was to be conducted with a revised protocol which reduces the number of genetically engineered cells that could be transferred into the patients and forbids therapy in boys before their first birthday. Gene therapy also resumed in the United States with similar restrictions.

The experience with gene therapy in boys with X-linked SCID has cre-

ated a terrible dilemma for parents, physicians, and authorities charged with the oversight of the research. Imagine yourself a parent of a little boy who has just been diagnosed with SCID. How would you weigh these facts? At this writing, 17 boys with X-linked SCID have undergone gene therapy, 10 at Necker and 7 in the United States and Britain. In all cases, the treatment for SCID has been successful, and the children have regained a functional immune system. Because no one knows how long the transplanted cells and their progeny will work, it is too soon to claim that they are cured of SCID. We must wait several years to see whether their reconstituted immune systems continue to produce functional cells.

Nevertheless, their status—some have been in remission for more than four years—justifies optimism. Until a few years ago, the diagnosis of X-linked SCID combined with the lack of a compatible donor was a death sentence. Today, parents can expect that affected children who undergo the gene therapy will in a few months have a competent immune system. On the other hand, 3 of the 17 (all treated at Necker) have developed leukemia (the most recent is different from the first 2), and 1 of the 3 little boys has died. The French have halted the program at Necker Hospital, and the United States has suspended its three trials. It will only permit gene therapy in boys who have undergone a bone marrow transplant that was unsuccessful. The British have reached a different conclusion about the risks and benefits and are continuing their program.

How does one balance the risk of death from SCID against the risk of death from leukemia? About 20% of boys who are diagnosed with X-linked SCID find a donor who is an appropriate match for bone marrow transplantation (which itself carries a small risk of death). For these children, it seems clear that the proper choice is transplantation. But what of the other 80%? For those who do not have the opportunity to undergo bone marrow transplantation, the risk of death is extremely high. It is impossible for parents not to exist in a state of chronic fear. Long before it kills, the disease takes a great toll.

Should parents of children who do not have a potential bone marrow donor be permitted to consent to gene therapy even though it may carry a 20% risk that the child will later develop a potentially fatal leukemia? Arguments against undergoing gene therapy for SCID include the possibilities that (1) a perfectly matched bone marrow donor will be found, (2) there will be advances in using tissue from imperfectly matched donors, and (3) there will be further advances in gene therapy, especially in regard to regulating the insertion of the gene vectors (thus, reducing the risk of leukemia). The major argument in favor of going forward with gene therapy centers on the daily risk to the child of contracting a fatal infection. But there is another. The 2 children who developed leukemia due to an insertion in the LMO2 gene underwent treatment before the age of four months. Given the age distribution of the 10 children, this is an unlikely event. Because of their knowledge of how the immune system matures, some researchers think that older children are at lower risk of contracting this form of T-cell leukemia. Even in the face of a daily threat of serious infection, the right clinical course may be to wait until the child is at least one year old.

Ethics is not a statistical science. The central principle before each set of parents (the children are far too young to exercise choice) is to decide what is in the best interests of the child. It is hard to make judgments on limited facts, but sometimes we have to. It seems to me that it would be right to forbid gene therapy for children with SCID who are younger than the age that a consensus of experts decides marks a reduction in risk for developing leukemia after gene therapy. With good medical care, most can be kept alive until they reach that age (which is likely to be about one year). I am not comfortable with the decision in the United States to forbid gene therapy to those who had not first tried bone marrow transplantation. Since both therapeutic options are fraught with danger, I think the parents of affected children should have the right to choose.

The saga of gene therapy for X-linked SCID is, despite the setback due to the development of leukemia in two patients, a remarkable success story. First, it was based on solid science and comprehensive animal studies. Second, the physician-researchers acted promptly and appropriately when problems arose. Third, the regulatory bodies responded with appropriate caution but, mindful of the needs of the patients and their families, they worked assiduously to find a means to safely reopen access to treatment. Fourth, scientists probed the mystery of the leukemia and came up with a very plausible explanation and a game plan to circumvent the risk in the future.

The careful investigation of every infant who presents with an appar-

ent genetic disease that is compromising the immune system has led researchers to delineate at least 11 different forms of SCID. In turn, they have so far found mutations in 10 different genes that can cause SCID. This is almost invariably the pattern in our history of delineating several thousand human genetic diseases. The more meticulously we study the patient's clinical condition and his DNA, the more likely we are to describe a new cause for the illness. More research will refine the therapeutic approach. In 2005, researchers reported that gene therapy had failed in the treatment of two older children with a slightly milder form of the disease, suggesting that as-yet-unknown age-dependent factors were important in this approach.

The possibility that gene therapy will become the treatment of choice for a number of similar disorders of the immune system is good. In addition to the successful treatment of most of the boys with SCID, researchers have successfully used gene therapy to treat four children with adenosine deaminase deficiency (ADA). When described by doctors in 1972, ADA became the first of the steadily growing number of immunodeficiency diseases. It is an autosomal recessive disorder that affects equal numbers of boys and girls and accounts for most of the non-SCID patients. Kids with ADA have an illness that is remarkably similar to SCID. Until recently, most died before the age of five from infection. Researchers have been attempting gene therapy in ADA patients for a decade. Follow-up studies have demonstrated that the genetically engineered T cells persist in the patients for at least that long. Over the last few years, improved techniques in the delivery of the engineered cells have resulted in substantial improvement in immune function without serious side effects. Emboldened by success, expert physicians are beginning gene therapy trials for a growing number of immune deficiency diseases. In addition to experiments involving patients with well-known single-gene disorders such as muscular dystrophy and cystic fibrosis, researchers are working on using gene therapy to treat a form of brain cancer and heart disease.

Only about 50–100 boys are born with X-linked SCID in the United States each year. Given the host of far more common health problems faced by children, the triumph over this disorder may not seem great. However, the successful use of gene therapy to treat (perhaps cure) SCID means that research to expand its applications could grow quickly. It is

very likely that patients with several less esoteric genetic disorders such as hemophilia, muscular dystrophy, and cystic fibrosis, disorders affecting many thousands of children, will (over the next decade) benefit from this wonderful new field.