Autoimmune Disease: New Evidence About Lupus

Systemic lupus erythematosus (SLE or lupus), which was once considered a rare disease, is now thought to afflict as many as 500,000 people, with perhaps 50,000 new cases diagnosed every year. Nevertheless, relatively few persons outside the biomedical community have heard of SLE. Biomedical investigators, however, have long been interested in this complex disease both for its own sake and because they hope that understanding lupus will enable them to understand related conditions, including rheumatoid arthritis, which affects an additional 5 million persons.

Lupus and rheumatoid arthritis are among the autoimmune diseases. In such conditions, the immune system, which normally plays a beneficial role in warding off bacterial and viral invasions and possibly in preventing the growth of tumor cells, goes awry and begins to attack the body's own tissues. In rheumatoid arthritis, it is the joints that are the targets of the attack; they become inflamed, begin to degenerate, and may eventually be immobilized.

In SLE, the immune system may attack any organ or system. Those most frequently affected are the skin, joints, kidneys, brain, lungs, and heart. About one-quarter of the patients may suffer severe kidney damage and one-third have brain damage. For these individuals, the prognosis is bad. Others may have a relatively mild disease. In general, SLE is a chronic illness with periods of remission and relapse. Sunlight can trigger exacerbations in an unknown manner.

Finding out what causes the aberrant immune responses of SLE patients has not been easy. Recent evidence indicates that genetic susceptibility, viral infection, and faulty control of the immune system all interact in the etiology of the disease, but the primary cause—if there is only one—has remained elusive. Current research has, however, delineated some of the mechanisms that produce the pathological changes of lupus. And these findings are contributing to improved diagnosis of a condition that is often hard to identify because of its protean character.

Much of the information about SLE comes not only from human patients but from studies on New Zealand black (NZB) mice and hybrids between NZB and New Zealand white mice. These animals spontaneously develop a condition that closely resembles human SLE. One aspect of the mouse disease is glomerulonephritis (inflammation of the nephrons or filtering units of the kidney) very much like that causing kidney damage in humans. Moreover, the fact that the glomerulonephritis is more severe in female mice than in males is also analogous to the human situation—females constitute approximately 80 percent of SLE patients.

Another characteristic of NZB and hybrid mice—and of human SLE patients—is that they have antinuclear antibodies (antibodies against DNA-containing materials) in their blood. In fact, Frank Dixon and his colleagues at Scripps Clinic and Research Foundation showed in the mid-1960's that complexes of these antibodies with their antigens deposit in the mouse kidneys. Some components of the complement system are also present in the deposits. The complement system consists of several blood proteins that interact with antibody-antigen complexes to produce inflammation. The investigators think that deposition of the immune complexes in the kidneys triggers the damaging inflammatory process. Moreover, Henry Kunkel, David Koffler, and their colleagues at Rockefeller University have shown that the kidneys of human SLE patients contain deposits of complexes of DNA and antibodies against DNA.

The source of the nucleic acids eliciting production of the antibodies is unknown. They could be released from damaged cells, possibly as a result of viral infection. Or they could themselves be of viral origin. In any event, several investigators have linked viruses with the development of the mouse disease, and there are also reports implicating them in the etiology of human SLE. The viruses thus implicated are of the C type. Type C viruses contain RNA as their genetic material; they cause a number of mammalian and avian cancers and may possibly be involved in the development of certain human cancers, including acute myelocytic leukemia.

Some of the early evidence suggesting that viruses might cause the SLE-like syndrome of mice came from the laboratories of Robert Mellors of the Hospital for Special Surgery and Cornell University Medical College and June East of the Imperial Cancer Research Fund Laboratories in London. According to these investigators, electron microscopic examination revealed particles with the structure of type C viruses in the tissues of NZB mice. Consequently, they proposed that a type C virus produces the mouse autoimmune disease and also the cancers that the animals may develop. The cancers are usually malignant lymphomas in which abnormal numbers of lymphocytes are produced by the spleen and lymph nodes.

Recently, Jay Levy and his colleagues at the University of California Medical School in San Francisco identified a new kind of type C virus produced by NZB mice and all other strains of house mice examined. It is not the same as the mouse leukemia virus originally described by Ludwik Gross. The virus discovered by Levy is an endogenous one that is transmitted genetically from parent to progeny in the egg and sperm. This means that copies of the viral genome must be incorporated into the cellular genome. (Type C viruses have the enzyme reverse transcriptase for copying RNA into DNA.) The virus has to be transmitted genetically because it cannot infect mouse cells directly. It can, however, infect and reproduce in cells of other animal species. For this reason, Levy has called it a xenotropic virus.

Many investigators, including Levy, think that xenotropic viruses play a role in normal processes such as cell differentiation and embryo development. But in some circumstances they may also be involved in the genesis of autoimmunity and tumors. According to Levy, almost every kind of NZB mouse cell, even cells taken from early embryos, produce large quantities of the xenotropic virus. Although other mouse strains harbor the virus, these animals produce much less of it than do NZB mice.

Levy suggests that a genetic defect of NZB mice permits enhanced expression of the virus and that this in turn results in autoimmune disease. The pathological changes could be due to generation of antibodies against viral components that could combine with the components to form immune complexes that deposit in the kidneys and other organs. Another possibility is that the presence of viral antigens on cell surfaces provokes an

immunological attack on the cells. This attack might also occur if viral expression somehow altered cellular antigens so that they were no longer recognized as "self."

Investigators have also used immunological techniques to try to pin down the role of viruses in SLE. These techniques involve making antiserums to virus components and using the antiserums to probe for the components in cells. With Robert Huebner and Tadao Aoki of the National Cancer Institute, Mellors identified antigens characteristics of type C viruses in thymuses, spleens, and kidneys of NZB mice as young as 5 days of age. The investigators once thought that the antigens were characteristic of the Gross leukemia virus but further investigations have suggested that the antigens may be those of the xenotropic virus described by Levy.

In any event, one of the viral antigens appears in the blood of mice 3 months of age or older—approximately 1 to 2 months before the onset of glomerulonephritis. As the symptoms of this condition appear, the antigen begins to disappear from the blood. At the same time, the incidence of antibody against the viral antigen increases. The investigators hypothesize that the presence of the viral antigen stimulates the immune system of the mice to make antibody against it. The formation of large quantities of antigen-antibody complexes overwhelms the normal mechanism for removal of such complexes from the blood and some of them deposit in the kidneys; excess antibody remains in the blood stream.

Several investigators, including Dixon and Richard Lerner, also at Scripps, have found that type C viruses, when injected into immunologically normal mice, induce glomerulonephritis similar to that found in NZB mice. According to Lerner, viruses stimulate the production of antibodies against nuclear material and viral components. These antibodies can then end up in immune complexes in the kidney. The relative role of viral antigens and antinuclear antibodies in forming the complexes is unclear from these and other studies. Both have been found in kidneys from mice and from human patients and both may contribute to the damage.

Several investigators have evidence that RNA viruses are implicated in the etiology of human SLE just as they are in the mouse disease. For example, Mette Strand and Thomas August of Albert Einstein College of Medicine detected antigens related to those of type C viruses in the kidneys of certain SLE patients. And Robert Schwartz and his colleagues at Tufts University Medical School found type C viral antigens in lymphocytes from some individuals with SLE.

Recently, Robert Mellors and Jane Mellors, also of the Hospital for Special Surgery, detected an antigen in the kidney lesions of a patient who died of SLE. They showed that the antigen was similar to one of the structural components of an endogenous type C virus of primates. None of the ten control kidneys had the antigen.

Further evidence for the hypothesis that a type C virus is involved in the development of human SLE comes from the laboratory of Sandra Panem and Werner Kirsten at the University of Chicago Medical School. They prepared an antiserum against a type C virus that they had previously recovered from a line of cultured human fibroblasts. The investigators have evidence that the virus is related to one of the type C viruses of primates and think that it is possibly of human origin. With the antiserum Panem and Kirsten detected virus-related antigens in the kidneys of 11 SLE victims but in none of the control kidneys. Kirsten says that so far the results of the studies have been almost too good to be true and they are still looking for exceptions to them. These would be SLE kidneys without the antigens or control kidneys with them.

The Immune System and SLE

Current investigations of the immune defects of human SLE patients and NZB mice have focused on the function of T lymphocytes. These cells are responsible for cell-mediated immunity and also for regulating antibody production by B cells. Both the human and mouse autoimmune conditions are characterized by defective cellular immunity and by the production of unusual antibodies such as those against nucleic acids and various body tissues. In addition, unusually large quantities of a number of normally occurring antibodies are present. Decreased numbers of T cells could account for both these phenomena.

The experiments of Norman Talal of the University of California and the Veterans Administration Hospital in San Francisco and Alfred Steinberg of the National Institute of Arthritis, Metabolism, and Digestive Diseases showed that NZB mice progressively lose their suppressor T cells as they age (*Science*, 18 April 1975, p. 245). Since suppressor T cells suppress antibody production in response to some antigens, their loss could help to explain the abnormal antibody production of NZB mice and possibly of human SLE patients. Immunologically normal mice lose suppressor activity with age but not nearly as early in life as do the NZB animals.

Levy, among others, has hypothesized that viral antigens or cellular antigens altered as a result of viral expression if present on thymus cells, could lead to destruction of these cells by the immune system. There would be a consequent deficiency of T lymphocytes since the thymus gland is needed for the normal maturation of these cells. Investigators, including Edward Boyse of Sloan Kettering Memorial Institute, have shown that a surface component of thymus cells that is associated with certain developmental stages of the cells is at least antigenically similar to a major glycoprotein constituent of type C viruses. This might be one target of attack. Moreover, Lerner has identified an antigen related to this same virus component in tissues of NZB mice.

Both NZB mice and human SLE patients have antibodies directed against thymus cells or T lymphocytes that may participate in the destruction of the cells. Mellors has detected such antibodies in the mice. Steinberg and his colleagues noted that patients with active SLE have reduced quantities of one subpopulation of T cells (as identified by their fractionation procedure). Incubation of serum from the patients with cells from normal donors results in a decrease in the same subpopulation. The investigators located the material producing this effect in the antibody fraction of the SLE serum.

The thymus gland secretes hormones that are needed for the maturation of functional T cells. Talal showed that weekly injections of thymosin, a hormone preparation from bovine thymuses (*Science*, 28 March 1975, p. 1183), into NZB mice could preserve their suppressor T cell functions. However, when he attempted to use the material to prevent or cure the animals' autoimmune disease, he found that administration to young mice delayed but did not prevent onset of the disease. When given to older mice with abnormalities already established, the thymosin had no effect.

On the other hand, Steinberg says that biweekly injections of young thymus cells (the NZB donor mice were less than 2 weeks old) did prevent the development of the hemolytic anemia of NZB mice until the injections were discontinued when the animals were 1 year old. (Human SLE patients may also acquire this kind of anemia, which is due to immunological attack on red cells.) Control animals developed the anemia between 4 and 5 months of age.

Steinberg hypothesizes that the presence of suppressor T cells in the injected

material may prevent the development of the antibodies participating in red cell destruction. Many investigators think that suppressor T cells exert their effects by releasing soluble suppressor substances. If these could be identified in humans, they might provide a method for remedying a primary defect of SLE.

In contrast to the findings about suppressor effects, which appear to decrease prematurely, Talal and his colleagues showed that other functions of T cells develop faster than expected. According to these investigators, the activity of helper T cells that are required for formation of antibodies against some antigens may be increased in young New Zealand mice. Talal says that the current state of knowledge about helper and suppressor T cells does not permit the determination of whether there is a real increase in helper activity or whether it merely appears high because suppressor activity is low.

Why autoimmune disease is more prevalent and serious in females than in males is another puzzling question. Talal and his colleagues have examined the development of antibodies against DNA and RNA in young male and female hybrid mice in order to determine what causes this difference. They found that at 2 and 4 weeks of age both males and females have low concentrations of antibodies against the nucleic acids. Talal thinks that the early formation of these antibodies could reflect a loss of suppressor cells. The results of these investigations further suggest that formation of antibodies, especially against RNA, follows a normal developmental sequence but that it is accelerated in female mice as compared to males. Other investigators have shown that helper T cells are involved in regulating the development of antibody responses, and Talal hypothesizes that females have excessive helper cell functions.

Talal now has evidence that the sex hormones affect T cell regulation of antibody responses and are thus the mediators of these immunological differences between males and females. Castration of 2-week-old NZB male mice changes their antibody patterns so that they resemble those of females. This would indicate that the male sex hormones tend to protect against autoimmunity.

Castration of female mice has little effect on the appearance of antibodies against DNA but does result in markedly decreased production of antibodies against RNA. Moreover, it prevents the accelerated development of antibody responses to RNA. Thus, female hormones also affect antibody responses. That even normal females generally produce more antibodies than males may be a reflection of hormonal differences. Since the effect of removing the thymus glands of both males and females are similar to those of castration, Talal hypothesizes that the sex hormones produce their effects by acting on thymic cells.

Genetic susceptibility appears to predispose NZB or the hybrid mice to autoimmune disease and investigators think that the same may be true for humans. Sorting out the relative influence of heredity and environment, which includes exposure to viruses, can be difficult. However, studies of twins indicate that heredity does play a major role in the etiology of SLE. According to Charles Christian, Sidney Block, and their colleagues at the Hospital for Special Surgery, both members of four pairs of identical twins had clinical SLE. Only one member of each of three additional pairs of identical twins had overt disease, but two of the apparently well twins had immunological abnormalities like those seen in lupus. The study included three pairs of fraternal twins, each consisting of one twin with SLE and one without overt symptoms, although one of these latter individuals did have immunological abnormalities.

Familial Incidence of SLE

There is disagreement about the incidence of SLE among first-degree blood relatives (parents, brothers, and sisters) of lupus patients, who would normally share the same environment with the patient, but the incidence is significantly lower than that in identical twins. One study indicates that such persons are no more likely to get the disease than are members of the general population. (Estimates of the overall incidence range from 5 to 50 cases per 100,000 persons, with part of the uncertainty due to difficulty in diagnosing the condition.) Another study indicates that the incidence among first-degree blood relatives is 1.5 percent. Thus it appears that the effects of heredity surpass those of environment but the fact that not all of the identical twins get overt clinical disease indicates that additional factors, perhaps viruses, are still necessary for SLE to develop.

Further evidence for a genetic basis for lupus comes from Peter Schur and his colleagues at Robert B. Brigham Hospital in Boston. They have evidence that an inherited deficiency of one of the components of the complement system is more common in patients with SLE and the juvenile form of rheumatoid arthritis than in controls. According to Schur, 6 percent of SLE patients and almost 4 percent of those with juvenile rheumatoid arthritis were deficient in the second complement component whereas only 1.2 percent of the controls were. They do not know how this deficiency could predispose individuals to lupus, although faulty handling of viral infections might be involved.

Other components of the complement system may also be decreased in SLE, but these deficiences do not appear to be genetically controlled. Rather, they seem to result from the disease process. Schur has found that low levels of certain complement components plus high concentrations of antibodies to DNA are always associated with active disease, especially active renal disease. He thinks that the changes represent removal of complement components from the blood by their binding to the immune complexes that deposit in the kidney.

Several investigators have noted the association between active SLE and high concentrations of the antinuclear antibodies. Testing for these antibodies has become one of the more accurate methods of diagnosing lupus, which can be difficult because so many different organs may be affected and the symptoms resemble those of other diseases. Some symptoms, such as a butterfly-shaped rash across the cheekbones and over the nose and a positive test for LE cells (an unusual kind of leukocyte) are considered quite characteristic of SLE, but they are not found in all patients. However, most SLE patients have high concentrations of antinuclear antibodies, which are found only rarely in other conditions.

Schur thinks that periodic determinations of the concentrations of the complement components and antinuclear antibodies in the blood of SLE patients might permit physicians to predict relapses so that treatment could be initiated before serious and irreversible organ damage ensues. During the course of one study, some 32 patients suffered relapses. According to Schur, 22 of these individuals had a 50 percent decline in the concentration of complement components at the time the relapse began.

Investigators have also focused on possible associations between histocompatibility antigens and SLE in an attempt to pin down the causes of the disease. Histocompatibility antigens, which are located on cell surfaces, are the antigens that elicit rejection of transplanted organs by the immune system. Investigators are interested in them with regard to SLE because they may either be directly involved in provoking autoimmunity or they may be indirectly

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involved because of their genetic association with immune response genes. The genes for histocompatibility antigens and those controlling immune responses to a number of antigens are closely linked on the same chromosome. Faulty control of immune responses has been strongly implicated in the etiology of lupus. Thus, association of the disease with a particular histocompatibility antigen might actually reflect linkage with an immune response gene whose expression produces the faulty immune responses of SLE.

Some investigators, such as Lawrence Shulman of Johns Hopkins University Medical School, have found associations between SLE in humans and particular histocompatibility antigens. Not all of them have implicated the same antigens, however. In the Johns Hopkins study, two of the antigens, HL-A1 and HL-A8, were found more frequently in lupus patients than in controls. Approximately 20 percent of the patients had at least one of the antigens, with 12 percent having both. The comparable figures for the controls were less than 10 percent and 5 percent, respectively. Shulman says that association of these antigens with severe disease involving the kidney and brain is even greater.

To say that the etiology of SLE is complex is an understatement. Genetic susceptibility, viruses, and a defective immune system all appear to be involved. Since some type C viruses have been implicated in the development of cancers in addition to that of autoimmunity, Lerner and other investigators have hypothesized that autoimmunity may be the price of protection against virus-induced tumors. In other words, when the virus stimulates the immune system sufficiently to prevent tumor growth, autoimmunity results, either as a result of immune complex formation or by attack of the immune system on cells bearing viral antigens, or both. On the other hand, when the response of the immune system is weak, tumor growth can occur.

But SLE, although more common than once thought, is still a relatively rare disease in humans, if not in NZB mice. This implies that additional factors, possibly genetically determined and possibly involving abnormal immune responses, must also be considered. Some investigators even think that when the mystery of SLE is completely unraveled, the condition will turn out to be a complex of diseases with somewhat different etiologies.—JEAN L. MARX