Viruses and the Connective Tissue Diseases*

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A number of observations in the last few years have attracted attention to the possibility of viral infection in systemic lupus erythematosus (SLE). One of these is the occurrence of interwoven tubular structures, usually in the endothelial cells of the kidney but also in the lymphocytes and in fibroblasts when SLE skin fibroblasts are cultured. These tubular structures have resembled viruses (they were thought by their discoverers to be myxo- or paramyxoviruses), but it has been argued (1) that they are not viruses because of their size and appearance and because they have been produced in tissue cultures from subjects who do not have SLE; they occur in many other conditions which are not related to SLE. These tubular structures do not occur, however, in rheumatoid arthritis (RA). The conclusion, at this point, would seem to be that they are not viruses. Nevertheless, from their randomness and their odd appearance, it seems likely that we may be seeing the effects of a virus, perhaps a very common virus, on the endoplasmic reticulum of the cell.

There has been a group of papers in which the point has been made (2, 3, 4) that the titers of a number of viral antibodies (usually myxo- and paramyxoviruses) are somewhat elevated in patients with SLE. It has been argued that this simply represents the overall hyper-γ-globulinemia of SLE, although we have not noted the relationship with γ-globulin levels. It should be pointed out that there has been no outstanding elevation of any particular viral antibody titer. The antibody titer to measles, an RNA virus, has been consistently elevated to a significant degree. This may mean that antibody to this virus cross-reacts well with an unidentified RNA virus which is actually involved.

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Cellular Immunity in SLE. Hahn and coworkers (5) have reviewed the evidence for reduced delayed hypersensitivity in SLE. About half of investigators have noted decreased skin test reactions in this disease. Patients with SLE also tend to develop lymphomas somewhat more commonly than expected. Presumably these patients do not have adequate immunologic surveillance for rejection of lymphomas on the basis of diminished cellular immunity.

Regarding cellular immunity, it is known that thymus-derived lymphocytes undergo blastic transformation and produce lymphokines. When the T cell has been stimulated, it can also subserve the antigen to stimulate B lymphocytes (bone marrow-derived) to produce antibody.

With regard to the possible role of a virus in SLE and the fact that depressed cellular immunity seems to be present, it is fairly well accepted that virus infections, in general, decrease delayed hypersensitivity as measured by skin tests and by the response of the circulating T cells of the infected patient to mitogens like PHA and concanavalin. Also, if one adds a virus like rubella to lymphocyte cultures, the capacity of the T cells in that culture to undergo a response to mitogenic agents is diminished.

As is well known, the New Zealand Black (NZB) mouse, particularly its F_1 hybrid, is an excellent model for SLE. The NZB F_1 hybrid mouse has also been shown to have decreased cellular immunity (6). It has a decreased capacity to induce graft-versus-host disease in a recipient mouse strain. There is also a decreased responsiveness to mitogenic agents and a high incidence of lymphomas.

For unexplained reasons, T cells in man form rosettes with sheep red blood cells. These rosettes represent a measure of the T cells circulating in the blood. Wybran and Fudenberg (7), using this

method, found that patients with viral infections have reduced numbers of circulating T lymphocytes. In our laboratory, Drs. Hurd and Giuliano have observed decreased numbers of spontaneous rosette-forming cells in patients with SLE suggesting decreased cellular immunity. This could be a result of coating with an immunosuppressive globulin or a true deficiency of T cells.

Autoantibody Formation. It should be pointed out that in SLE, we are concerned not as much with T cell function as with the results of B cell activity since these cells produce autoantibodies such as anti-DNA, both double- and single-stranded, antinuclear antibody, anti-RNA, both double- and single-stranded, as well as other types of autoantibodies. Are such autoantibodies found in virus infections? Two conditions stand out—one, infectious mononucleosis, an EB virus infection, and the other, cytomegalovirus infection. In these conditions, we see antinuclear antibody, rheumatoid factor, mixed cryoglobulins, Coombs autoantibodies, and so forth.

The NZB mice are infected with murine leukemia viruses. This strain, unlike other strains, does not develop tolerance to the murine leukemia viruses and eventually develops antibodies to these viruses. These form immune complexes which circulate in the blood and in time the mice develop proteinuria. The immune complexes eventually deposit in the basement membrane of the kidney. If NZB F_1 hybrids are injected with nonleukemogenic viruses such as LCM, which is an RNA virus, or with polyoma, which is a DNA virus, antinuclear antibody is enhanced, the glomerulonephritis is aggravated and the mortality rate goes up (8). These viruses, it appears, exert an adjuvant effect in stimulating autoantibody formation. Nucleic acids are known to have adjuvant effects. Weight for weight, the RNA of the tubercle bacillus is as effective an adjuvant as the tubercle bacillus itself (9). Powell and Steinberg (10) have examined the adjuvant effects in the NZB-NZW mouse of poly I–poly C, a synthetic double-stranded RNA, and have found an increase in antibody, not only to double-stranded RNA, but also an increase to DNA. Cone and Johnson (11) have shown that poly A–poly U increased the antibody response against sheep red blood cells. One may speculate from these kinds of results that the viral nucleic acids may also have an adjuvant effect on both antibody and autoantibody formation.

It appears likely that if a virus were to be the cause of SLE, it would be so by affecting T lymphocyte function. In our laboratory (12), we have demonstrated that T cells produce a relatively low molecular weight factor, which has a stimulatory effect on antibody formation by B cells. Fialkow, Gilchrist and Allison (13) have shown that T cell stimulation can lead to autoantibody formation. F_1 hybrid mice injected with parental cells undergo a graft-versus-host reaction in which the donor T cells proliferate. These mice develop antinuclear antibody after each injection of T cells, presumably because of the elaboration of a helper factor by the proliferating T cells.

There is also evidence that a suppressor population of T cells exists which presumably inhibits autoantibody formation. The first evidence for suppressor T cells came from our laboratory (14) in a study of the effect of antilymphocyte globulin on the immune response of rabbits. When rats were treated with this agent, the antibody response to keyhole limpet hemocyanin was increased instead of reduced. This result has been interpreted by others (15) to indicate that there is a type of T cell whose function is to suppress the antibody response. Subsequently, Baker and co-workers (16) showed that there was an increased response to pneumococcal polysaccharide in mice upon treatment with antithymocyte serum.

Recently, Steinberg and co-workers (17) showed that neonatal thymectomy of NZB mice accelerated the formation of anti-DNA antibodies and that this change could be reversed by grafting these mice with one-week old thymuses, but not with ten-week old thymuses. Suppressor T cells presumably are lost in the NZB F_1 hybrid at an early age. This seems to be the cause of the aggravated form of the disease observed in thymectomized animals.

It is well established that autoantibodies develop in the aged (18). Phytohemagglutinin responsiveness, which is a measure of T cell function, also decreases. These changes may reflect a loss of suppressor T cells with age in man. When Teague and Friou (19) administered syngenic thymocytes from young mice to older mice who were producing antinuclear antibodies, these antibodies disappeared, suggesting that suppressor T cell function had been restored. The explanation for these phenomena, most clearly enunciated by Allison (20), is that we are dealing with a particular type of tolerance in the normal state which does not permit autoantibodies to form.

This tolerance resides in the T cell, as originally suggested by Weigle (21). The B cells which produce antoantibody are not tolerant and when they are stimulated by helper substance from helper T cells or freed from the suppressive action of suppressor T cells, they become free to synthesize autoantibody in the presence of autoantigen. In speculating about the role of a virus in autoimmune phenomena, one might suppose that an infected individual would have T cells sensitized to the causative virus, whatever virus that might be. This virus could then stimulate these sensitized T cells to produce a helper substance. As a result, potentially responsive (nontolerant) B cells would gain the capacity to produce autoantibodies. Thus, viruses might act as adjuvants for autoantibody formation by stimulating sensitized T cells to produce helper substances, which could then stimulate B cells to produce autoantibody in the presence of autoantigen. They could also interfere in some unexplained manner with the suppressor effect of suppressor T cells by producing a broad interference with T cell function since, as mentioned, T cell levels are, in fact, low in the virus diseases (7).

Another interesting line of evidence which should be mentioned comes from the studies of Lewis and co-workers (22) who have injected filtered suspensions of spleens of dogs with SLE to CA F_1 mice. These mice have, as a result, developed antinuclear antibodies. This finding suggests the presence of a filterable agent, probably a virus, in the affected donor dogs, having the capacity to produce antinuclear antibodies.

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