Report of Symposium
Viruses, arthritis, and connective tissue disorders*

A one-day Symposium on the putative role of virus infections in connective tissue diseases was organized by the Arthritis and Rheumatism Council and held at The Royal College of Physicians, London, on Friday, March 22, 1974. The speakers considered the strategy of virus infection, the various forms of host response, and the ways in which these can be altered. The immunopathological features of chronic virus infections were also reviewed. Examples of arthritis known to be induced by viruses were described and, finally, the evidence for virus infection in connective tissue diseases of unknown aetiology was discussed.

Session 1. Viruses and connective tissue diseases

Dr. M. Ziff (University of Texas Southwestern Medical School, Dallas, Texas, U.S.A.), Chairman, opened the session by considering the evidence for a viral aetiology in systemic lupus erythematosus (SLE), much of which is admittedly inconclusive. The microtubular structures detectable by electron microscopy in the endothelial cells of blood vessels, in renal glomeruli, and in blood mononuclear cells of patients with connective tissue diseases are not of proven viral origin. Similarly, the variable spectrum of raised antibody titres found in chronic liver disease, myasthenia gravis, and in autoimmune thyroiditis as well as in SLE, may simply reflect the general increase in serum γ-globulin. However, skin-test responses, lymphocyte transformation by phytohaemagglutinin in vitro, and the numbers of blood lymphocytes which form spontaneous rosettes with sheep erythrocytes are all reduced in SLE and it is tempting to attribute these findings to virus infection, since similar abnormalities are observed in acute infections by measles, influenza, varicella, poliomyelitis, and hepatitis viruses, and also in congenital rubella infection and infectious mononucleosis. Possibly virus could act by interfering with the function of T-lymphocytes. Moreover, this population is spontaneously depleted in ageing NZB mice with autoimmune disease, a process which is accelerated by deliberate infection with both leukaemic and nonleukaemic viruses. However, this strain also indicates the importance of genetic factors. The recent experiments of Dr. Schwartz and his colleagues implicating an RNA tumour virus in the pathogenesis of SLE in a colony of inbred dogs (Lewis, André-Schwartz, Harris, Hirsch, Black, and Schwartz, 1973) is the most striking evidence for a viral aetiology acting in conjunction with genetic factors. Nevertheless, despite many family studies, the evidence for a similar interplay of infective and genetic factors in human SLE is far from complete and often conflicting; but the wide range of autoantibodies associated with infectious mono-
nucleosis is an additional pointer to a mechanism by which virus infection, in this instance EB virus, could induce autoimmune disease by ablating T-lymphocyte control mechanisms. Professor A. P. Waterson (Royal Postgraduate Medical School, London) discussed the immunopathological consequences of hepatitis virus infections. Although a range of agents can induce hepatitis, we are particularly concerned with infections by the specific agents of infective hepatitis and serum hepatitis. The discovery of Australia antigen has given us the opportunity not only of identifying a marker for the agent responsible for serum hepatitis but also of interpreting the pathological consequences of infection in terms of the immunological response of the host. In particular, the Dane particle detectable by electron microscopy seems likely to be the fully infectious virion and therefore a particularly good marker of infection (Almeida, Rubinstein, and Stott, 1971). Serial ultrastructural observations allow the sequence of events to be followed whereby both Dane particles and conventional Australia antigen appear in the serum, after which specific antibody becomes attached to each antigen leading to its immune elimination. Arthritis may appear during the phase of antigen elimination, at which time serum complement is decreased and the serum itself becomes anticomplementary. The arthritis is not uncommonly accompanied by an urticarial eruption. An association between polyaerteritis and an immune reaction of Australia antigen has long been recognized. By way of illustration, Professor Waterson described a patient recently investigated at the Hammer smith Hospital who presented with an urticarial rash and muscle pains and who developed polyarteritis accompanied by circulating immune complexes between Australia antigen and IgM antibody in antigen excess. The circumstances under which antigen-antibody complexes give rise to such complications clearly need further study since these immunochemochemical events may be entirely asymptomatic. In the discussion Dr. Blumberg drew attention to the importance of genetic factors in the distribution of Australia antigen which could modify the outcome of infection in various populations. For example, the subtypes of antigen encountered in Oceania and the South-west Pacific region are different from those prevalent in the United States.

Professor E. G. L. Bywaters (Royal Postgraduate Medical School, London) described the clinical features of rubella arthritis, but pointed out that arthritis was also associated with other virus infections and in particular with hepatitis, poliomyelitis, infectious mononucleosis, variola, varicella, and mumps virus infections as well as members of the Coxackie and arbovirus groups. Rubella virus has long been recognized as an embryopathic agent, but in the fetus it causes osteitis, not synovitis. In outbreaks of rubella a high percentage of those affected develop arthritis and the joint involvement has a variable relation.

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to the rash. Arthritis may be the sole clinical manifestation of the disease, in which event the diagnosis must be supported by a rising titre of specific antibody. An important diagnostic feature of rubella arthritis is the predominance of mononuclear cells in synovial effusions (Chambers and Bywaters, 1963). It should be emphasized that in only one reported case has the disease left residual joint changes. Rubella arthritis is of importance because it gives us the opportunity of defining those factors which render the joints of individual patients susceptible to involvement by a recognized agent. These factors are largely undefined, and future studies should include the characterization of immunopathological events in the affected joints and a detailed comparative analysis of the immune response in patients with and without this complication.

A similar theme formed the content of the contribution by Professor J. A. Dudgeon (The Hospital for Sick Children, London), namely, individual susceptibility to arthritis after rubella vaccination. In some outbreaks this can be attributed to the characteristics of the vaccine employed and this is a particular propensity of vaccines passed in duck embryo cells (Cooper, Ziring, Weiss, Matters, and Krugman, 1969). Thus the strain of virus and the cells used for attenuation remain significant factors. However, host factors such as the increased susceptibility in those immunized after puberty are of critical importance. Arthritis lasts for 1 to 3 days or up to 7 days if the vaccine has been prepared in dog kidney cells, while there may be recurrences for up to one year. The knees and fingers are the sites most commonly affected. Virus has been recovered from the synovial fluid of inflamed joints, but the arthritis tends to be associated with a rising antibody titre at a time when the viremia is already subsiding. Specific antiviral IgG antibody may be confined to synovial effusions.

Session 2. Biology of viruses with special reference to persistence of infection

Dr. R. Dulbecco (Imperial Cancer Research Fund Laboratories, London), Chairman, introduced the second session by outlining the general mechanisms by which virus may persist in mammalian cells owing to a peculiarity of individual cells or of a population of cells. After incomplete replication of virus the pro-virus may persist in integrated form, but may subsequently regenerate and express some of its functions. Alternatively, virus may not be integrated, but the population may continue to carry the virus even though only a small percentage of cells are infected. Virus may not be cytocidal in such cells, but may bud off from the cell membrane, as with certain myxoviruses and paramyxoviruses. Furthermore, virus may persist in the cell population with only an occasional growth spurt as may, for instance, be seen when the cells are transferred to in vitro culture. Examples of reactivated infections are viruses of the adenovirus and herpes groups. In these instances it is not pro-virus which is activated, but only a small piece of the original virion. This brief review of virus persistence is of importance because it illustrates the many ways in which an altering balance between virus and host defence could lead to intermittent disease activity such as characterizes many of the connective tissue disorders.

Dr. D. A. J. Tyrrell (Clinical Research Centre, Harrow) stressed that not all virus infections are cytocidal for host cells. Many forms of interference are recognized which result in an incomplete cycle of virus replication. Interference has been particularly well studied in infections of the respiratory epithelium. Many mechanisms can account for such interference, but of special interest is the ability of defective agents such as influenza and poliomyelitis viruses to block the replication of homologous virus. Interferon and interferon-like factors which may be proteins or glycoproteins in nature primarily block the assembly of virus proteins, but in addition probably switch off many other virus-directed intracellular processes. Furthermore, interferon has other biological activities, notably an immunosuppressive action. The importance of interference phenomena in relation to the connective tissue disease is the recognition that virus infection may have widespread effects on a variety of biological processes. Furthermore, interference can be exploited as an indirect approach for identifying viral agents in this group of disorders. Nevertheless, such methods require great interpretative caution as exemplified by the failure of Dr. Tyrrell and his colleagues to confirm the evidence of Dr. Hamerman and his colleagues (Smith, Hamerman, Janis, and Habermann, 1974) for an interference phenomenon on rheumatoid tissues.

Professor C. A. C. Mims (Guy's Hospital Medical School, London) illustrated the variety of routes available for viral dissemination and the wide range of tissues in which virus can replicate. Examples include dorsal root-ganglia, muscle spindles, thymus, and endocardium in various viral infections in laboratory animals and even human red cells in infections by the Colorado tick virus. Furthermore, the range of permissive cells is still higher in tissues of the fetus or immature host. An important route of viral dissemination to the tissues is from the endothelial cells of blood vessels after their initial replication at this site. Thus, herpes simplex virus and arthropod-borne viruses invade the brain via this route. In anatomical terms, viruses with the capacity to replicate in blood vessels which supply joints have but a short distance to traverse before gaining access to the synovial membrane and other articular structures. Our knowledge of the manner in which virus infections are disseminated makes the concept of persistent virus infection of joints highly plausible.

Dr. A. M. Denman (Clinical Research Centre, Harrow) considered the possible interactions between lymphocytes and viruses. Lymphocyte populations are concerned with the recognition of viral antigens, the initiation of antibody synthesis, and cytotoxic reactions against virus-infected cells. Techniques are available for assaying these populations in different patient groups, though the study of replicating antigens poses special problems. Moreover, it is still unknown whether significant abnormalities may take the form of increased or diminished responsiveness. Paradoxically, lymphoid tissues are sites of virus replication in many infections, and cultures of stimulated lymphocytes support the replication of certain viruses (Wheelock and Toy, 1973). However, other viruses are directly inactivated by lymphocytes and even those viruses which normally replicate in cultures of stimulated lymphocytes commonly fail to do so when the cells are obtained from donors with proven virus diseases such as recurrent herpes simplex infections or in some connective
tissue diseases. Thus the interference phenomenon described by Dr. Tyrell can be used to show that lymphoid cells from patients with connective tissue diseases of putative viral aetiology show the characteristics of such cells in chronic diseases of proven viral aetiology.

Session 3. Host responses to virus infection

Dr. A. C. Allison (Clinical Research Centre, Harrow), Chairman, introduced the third session by pointing out the crucial role of genetic factors in determining the outcome of virus infections. On occasion these factors may be obvious, as in progressive measles or varicella zoster infections in patients with immunodeficiency syndromes involving defects in cell-mediated immunity. However, our understanding of most of the defects leading to persistent virus infections such as lymphocytic choriomeningitis (LCM) virus in mice is imperfect. Also ill-understood is the ability of many virus infections to alter normal immune regulatory mechanisms with the induction of autoimmune disease (Allison, 1973).

Professor P. J. Lachmann (Royal Postgraduate Medical School, London) pointed out the paradox whereby specific immunity in the form of antibody production may nevertheless fail to eliminate the virus responsible for persistent disease. Possible explanations include deficient cell-mediated immunity, antibody of low affinity, or a response to inappropriate antigens. Complement deficiencies are also of likely importance. Subacute sclerosing panencephalitis (SSPE) is an example of a disease caused by a persistent virus infection, namely, defective measles virus. Though the virus itself is atypical, the relative susceptibility to the disease of infants exposed to measles infections before the age of two years suggests that deficient immune responses may be implicated. Investigations at Hammersmith Hospital point to a defect in some modalities of cell-mediated immunity to measles antigen, but this defect is not confined to this antigen alone. However, in addition, the serum of patients with SSPE appears to block the ability of their own lymphocytes to destroy measles-infected target cells in vitro. Furthermore, cell-mediated immunity to other antigens, but not to measles antigen, is restored by transfer factor, indicating that the defective response to measles virus may be specific. Indicating the complexity of the problem, preliminary studies by Dr. Fazekas de St. Groth show that the measles antibody in patients with SSPE is mainly of low affinity. Glomerulonephritis is another disease associated with immunological abnormalities. In mesangial capillary glomerulonephritis low serum levels of complement and alternate pathway factors result from continued complement activation. This sequence is most readily explained by complex formation secondary to persistent viral antigenaemia resulting in glomerulonephritis. These clinical illustrations point to a number of mechanisms by which virus infections could persist and it is evident that simple explanations do not explain all the facts.

Dr. G. R. V. Hughes (Royal Postgraduate Medical School, London) stressed the frequency of immune complex disease in virus infections, citing examples in mice, mink, horses, and pigs. Immune complex glomerulonephritis is not always simply attributable to the deposition of antibody complexed to viral antigen, and in NZB x NZW mice, for example, 45% of antibody eluted from the kidneys consists of antibody to DNA. In SLE, antibodies are found to a variety of nucleic acid antigens. Recently much attention has been given to antibody to double-stranded RNA which occurs spontaneously in SLE, whereas in experimental animals only antibody to single-stranded RNA can be induced. However, the recent finding of antibody to single-stranded RNA in laboratory workers frequently exposed to material from SLE patients strengthens the suspicion that these serological abnormalities may result from viral infection. The availability of a method for assaying antibody to double-stranded RNA of virus origin has provided a new method for testing recent claims that this antibody is inhibited most efficiently by RNA of viral origin. In SLE this antibody is both IgG and IgM in nature, and, though it is not directed at transfer RNA, it does cross-react with RNA in virus-infected tissues particularly during the stage of virus replication. Clearly, serological studies of this sort will have an important bearing on the search for a viral aetiology for SLE.

Dr. B. G. Aichon (University of Bristol) reviewed the evidence implicating EB virus in immunoproliferative disease (Epstein and Aichon, 1973). While EB virus is clearly the aetiological agent in infectious mononucleosis, its precise role in Burkitt's lymphoma and nasopharyngeal carcinoma is still debated. EB virus infection in childhood usually causes seroconversion, but not clinical disease. In late adolescence about one-third to one-half of infected individuals develop infectious mononucleosis. The picture in older age groups is variable. The oncogenic potential of EB virus has been shown in several ways. A variety of serological, immunofluorescent, and ultrastructural techniques have demonstrated the presence of the virus or of EB virus-coded antigens in the malignant cells of Burkitt's lymphoma. Moreover, significant antibody titres to virus-coded cell membrane antigens have been shown in patients with this disease. In addition, EB virus will transform normal cells in vitro. Finally, EB virus induces tumours in South American monkeys. Cell-free preparations from these tumours transform autologous cells in vitro while the virus can be activated in the tumours themselves by treatment with bromodeoxyuridine. The importance of EB virus in connective tissue diseases generally remains to be established, but its role in infectious mononucleosis—a disease frequently accompanied by autoimmune phenomena—is indisputable.

Dr. R. L. Carter (Chester Beatty Research Institute, London) reviewed the serological abnormalities which accompany infectious mononucleosis. Heterophile antibodies are an invariable accompaniment, but other antibodies encountered include antibody to red cell I and i antigens, lymphocytotoxins, rheumatoid factor, antinuclear antibody, smooth muscle antibody, and Wassermann antibody. In addition, serum immunoglobulins are raised, while the blood and bone-marrow aspires show some increase in cells with Ig receptors. Histologically the primary feature is hyperplasia of lymphoid cells in T-dependent areas, but without any increase in plasma cells. The circulating atypical cells have normal chromosomes and do not carry detectable EB virus so that they can best be regarded as part of the immunological reaction to EB virus. Furthermore, their receptors are entirely different from the atypical B-lymphocyte characteristics of lymphoblastoid cell lines. However, a minority of the
circulating cells must be regarded as transformed cells since infectious mononucleosis blood injected into baby hamsters induces immunoblastomas which secrete human immunoglobulin. Possibly the proliferation of transformed B-cells in infectious mononucleosis is controlled by T-lymphocytes performing some form of regulatory function.

Session 4. Immunopathology of chronic virus infections

The whole of this session was devoted to a review by the Chairman, Dr. M. B. A. Oldstone (Scripps Clinic, La Jolla, California, U.S.A.), of recent work in the mechanisms of virus persistence, particularly in association with immune complex disease. The recognition of circulating immune complexes in persistent virus infections dates from experiments in which virus bound to serum y-globulin could be precipitated by treating the serum specifically with antibody to y-globulin. Furthermore, examination of the kidneys by immunofluorescent techniques combined with elution studies showed that these complexes are deposited in renal glomeruli. In order to show viral antigen in sites such as the kidneys or blood vessels it may first be necessary to elute Ig deposits. Leukaemogenic viruses initiate a similar process. In AKR mice, antibody to viral envelope protein and reverse transcriptase can be eluted from the kidneys. Moreover, Ig of unknown specificity has been eluted from the kidneys of a large proportion of human cancer patients.

In lymphocytic choriomeningitis disease of mice, immune complexes similar to those encountered in the kidneys can be shown in the choroid plexus, whereas such deposits are lacking in scrapie, a virus infection of the central nervous system which does not elicit any detectable antibody response. Identical lesions can be detected in the choroid plexus of NZB × NZW hybrid mice and in human SLE. Transfer experiments with cell-free filtrates from NZB mice tissues suggest that a distinct endogenous RNA tumour virus may account for many disease features of this strain, including glomerulonephritis. Furthermore, in human disease, good evidence of immune complex disease with renal deposition has been obtained in serum hepatitis, SSPE, infectious mononucleosis, dengue, and Burkitt's lymphoma. In the latter condition, antibody to several EB virus antigens has been eluted from the kidneys.

Many mechanisms for the destruction of virus-infected cells have been detected in vitro. However, in vivo infected cells evade immune destruction. This may be dependent in part on the rate of turnover of such cells since rapidly proliferating cells are relatively resistant to lysis by antibody and complement. This resistance is not due to poor attachment of complement components or antibody. Experiments with HeLa cells infected by measles virus in vitro show that these cells are highly susceptible to antibody-induced complement lysis when viral antigens are regularly expressed over the cell surface. However, under appropriate conditions the viral antigens in combination with antibody become capped at one pole of the cell followed by extrusion of the complex into the medium. Such cells lack virus-specific surface antigens and resist lysis by antibody and complement. Cell-bound antibody may have a similar effect by preventing the expression of viral antigens at the cell surface. The capping phenomenon is prevented by various inhibitors of cell metabolism and is not produced by Fab fragments of antiviral antibody.

It is noteworthy that intracellular bodies may be seen ultrastructurally in these manipulated cultures which resemble those observed in the lymphocytes of patients with SSPE. Clearly these in vitro studies will provide insight into the mechanisms by which virus-infected cells may escape destruction by the immunological defences of the host.

References