Memoranda

Virus-associated immunopathology: animal models and implications for human disease *

2. Cell-mediated immunity, autoimmune diseases, genetics, and implications for clinical research

Part 2 of this memorandum describes further mechanisms whereby the interaction of a virus with the host's immune system may lead to tissue damage. Cell-mediated immunity plays a vital role in promoting recovery from virus infections, but under some circumstances tissue damage may be caused by the reaction of immune cells with viral antigens. When mice are infected with lymphocytic choriomeningitis virus neonatally or as adults while receiving immunosuppressive drugs, widespread invasion of cells is seen but there is little overt disease. If, however, normal adults are infected or if immune cells are transfused into tolerant mice, cell injury and death follow. Viruses have long been suspected of contributing to the pathogenesis of autoimmune diseases. Antibodies directed against normal cell constituents have been reported in several virus infections. Viruses may conceivably unmask or release host antigens, alter host antigens and act as " helper determinants ", or perhaps in other ways provoke immune responses against normal body constituents. The immunopathological manifestations caused by viruses may also be influenced by the host's genetic makeup. Certain observations indicate that, in addition to controlling susceptibility to virus infection, genetic factors partly determine the effectiveness of the immune response. The memorandum calls attention to the possible implications of these concepts and findings for clinical research. Some of the diseases of animals and man that serve as models for studies of virus-associated immunopathology are briefly described.

Part 1 of this memorandum on viral immunopathology (*Bull. Wld Hlth Org.*, 1972) dealt with the direct effects of viruses on the immune system and their role in immune-complex disease and antibody-mediated immunological injury. In Part 2 the relationship of viruses to cell-mediated immunity and autoimmune diseases is examined, the genetic aspects of the host's response to virus infection are reviewed, and some implications of the new concepts and techniques in viral immunopathology for clinical research are explored. Annex 1 describes a number of animal and human disease models in use in this field.

CELL-MEDIATED IMMUNITY

There is increasing evidence that in some virus infections the cell-mediated immune response plays a central role in recovery from infection (Allison, 1972; Blanden, 1971; Glasgow, 1970; WHO Scientific Group on Cell-Mediated Immune Responses, 1969), promoting the sterilization of tissues and the resolution of lesions. Under some circumstances, however, the reaction of immune cells with viral antigens, either free or on the surface of infected cells, may cause tissue damage. The laboratory model of cell-mediated immunopathology that has received the most study is lymphocytic choriomeningitis virus (LCMV) infection of mice (Oldstone & Dixon, 1970a).

LCMV-induced immunopathological damage

In adult mice infected with LCMV, viral replication occurs in many tissues, including the viscera and meninges. The infection is noncytopathic, and the animals remain free of clinical signs until the onset of an immune response 5-7 days after infection. Associated with this response are inflammatory cell infiltrations occurring at the visceral and meningeal sites of viral replication and leading to pathological changes, overt clinical signs, and death. Inhibition of the immune response by neonatal thymectomy,

irradiation, or the administration of antilymphocyte serum (ALS) (Mims & Tosolini, 1969; Wiktor & Koprowski, unpublished data) or cytotoxic drugs can prevent the acute disease. Mice infected with LCMV neonatally or congenitally also develop widespread infection but their immune response to the virus is poor. No clinical signs are seen in these animals during the early phase of the infection [1] and late in life chronic glomerulonephritis develops as a result of the deposition of virus-antibody complexes in the kidney (see Bull. Wld Hlth Org., 1972).

Attempts have been made to determine whether it is the antibody-mediated or the cell-mediated component of the immune response that causes the pathological changes in mice infected as adults with LCMV. In vitro experiments have shown that when spleen cells from LCMV-immune mice are added to LCMV-infected cells, the latter will be destroyed (Lundstedt, 1969). Cell destruction is probably mediated by a cytotoxic factor that is released when spleen cells from LCMV-immune mice interact with LCMV or LCMV-infected mouse fibroblasts (Oldstone & Dixon, 1970b).

The design of the corresponding in vivo experiments was as follows. Cyclophosphamide [2] was given to adult mice infected with LCMV (Cole et al., 1972), thus inhibiting the immune response, and a persistent clinically inapparent infection was established. When immune spleen cells were injected into these mice, the animals died and acute pathological changes comparable to these occurring in the natural infection were seen. Since the transfusion of immune serum produced less severe pathological changes, it was concluded that the injected cells had reacted with infected host cells to create a cell-mediated immunopathological condition. In congenitally infected carrier mice, in contrast, the transfer of immune spleen cells failed to

yield any clinical or histologic evidence of tissue damage. High titres of neutralizing and complement-fixing antibody were produced in the recipient carrier mice so that, under these circumstances too, the animals remained free of clinical signs. Other experiments, however, have shown that LCMV antibody plus complement is cytotoxic for LCMV-infected cells in vitro (Oldstone & Dixon, 1971a), and tissue lesions have been detected after the injection of large amounts of antibody into carrier animals. The possible immunopathological action of antibody was also suggested by the finding that complement depletion by means of cobra venom made LCMV considerably less lethal for adult mice. However, mice deficient in the C5 component of complement showed normal susceptibility to LCMV.

In summary, these results suggest that cell-mediated immunity may be an important factor in LCMV-induced immunopathological injury, although they do not rule out the possibility that antibody may also play a role.

Evidence of cell-mediated immunity in other virus infections

Certain experiments have indicated that cells persistently infected with measles or mumps virus (Speel et al., 1968) may be destroyed in vitro by incubation with spleen cells obtained from specifically immunized animals, but these studies need to be confirmed and expanded. It has also been suggested that cell-mediated immune responses may be responsible for the rashes associated with certain infections (e.g., measles) and might account for the skin lesions seen in rabbits infected with Shope fibroma (Tompkins et al., 1970) and rabbit pox viruses. Indirect evidence from patients with immunologic deficiencies also points to the importance of cell-mediated immunity in certain virus infections, but in others (e.g., enterovirus infections) cell-mediated immunity may be relatively insignificant.

There is some evidence that sensitized lymphocytes after reacting specifically with viral antigen may release biological mediators, such as migration inhibition factor (MIF), lymphotoxin, interferon, and factors chemotactic for polymorphonuclear and mononuclear leucocytes. Some mediators also activate macrophages, which will then show an increased capacity to take up and kill bacteria, protozoa, and possibly viruses. In addition, activated macrophages may interact with viral antigens on the surface of infected cells and in this way, perhaps with the help

---

[1] In addition to LCMV, some other microorganisms are known to produce less severe clinical signs in newborn than in older animals, e.g., the agents of many protozoal diseases and of heartwater. The latter is caused by a mycoplasma-like organism that grows in vascular endothelium and other sites. Infections of very young lambs or calves cause only mild clinical signs, even when numerous organisms are present for many months, whereas infections of older animals are severe and often fatal. The transfer of lymphocytes from old to young infected animals precipitates overt signs of disease in the latter. Although there may be other explanations—including maternal antibody—for the relative insusceptibility of young animals to some infectious diseases, the possible role of immunopathological reactions in adults with the disease should always be borne in mind.

[2] 2-bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide.

of cell-bound antibody, may play a role in the host's defence against virus infections (Tompkins et al., 1970) while at the same time contributing to the production of lesions (Allison, 1972).

Recommendations

(1) *In vitro* experiments to test for cell-mediated cytotoxicity of the type described above should be carried out in more virus infections. Caution should be exercised in interpreting negative results, however, because much may depend on the type of infected target cell used.

(2) The distribution and density of viral antigens on the surface of infected cells should be studied thoroughly, since these are the targets of cell-mediated immunopathology. Immunofluorescence techniques are relatively simple to use; alternatively, the topographic localization of antigen can be elegantly studied (Aoki et al., 1970) at the ultrastructural level by the use of reconstituted antibody molecules reacting with both viral antigen and marker particles.

(3) Since lysis of cells by sensitized lymphocytes might be prevented by a " blocking antibody " adsorbed on the surface of the target cells or by antigen–antibody complexes adsorbed on the sensitized lymphocytes or target cells (Hellström & Hellström, 1970), it would be desirable to investigate this phenomenon in virus infections where cell-mediated immunity may be involved.

(4) Sensitized lymphocytes may exert much of their protective or other immunologic effects by recruiting activated macrophages into infected areas. Further studies are therefore needed of the role played by macrophages not only in the expression and pathological consequences of cell-mediated immunity but also in the induction of the immune response to virus infections.

(5) Attempts should be made to assess the importance of cell-mediated immune responses in the pathogenesis of natural infections of animals. A useful approach would be to treat animals with cyclophosphamide, a drug that suppresses both antibody- and cell-mediated immunity (Cole et al., 1972), and then infect them with the virus to be studied. If cyclophosphamide treatment were found to decrease the pathogenicity of the infection without affecting viral growth in the tissues, then the respective parts played by immune cells and antibody could be investigated. Infected cyclophosphamide-treated animals could be given either cells or serum from normal or immune donors and the ensuing pathological changes and disease could then be recorded.

(6) Cells found to be immunocompetent, as shown by blast transformation after exposure to virus, should be transferred to cyclophosphamide-treated animals as in (5) with a view towards elucidating their role.

(7) Since the mechanism of recovery from some infections, notably human hepatitis, is still obscure, an analysis of cell-mediated immunity against specific antigens should be made. One approach would be to obtain lymphocytes (Rosenberg et al., unpublished data) from patients recovering from hepatitis, expose the cells *in vitro* to Australia antigen, and determine whether they undergo blast transformation.

AUTOIMMUNE DISEASES [1]

Autoimmunity is the general term used to describe an immune response, either antibody- or cell-mediated, against normal body constituents. The presence of autoantibodies to certain host antigens, such as thyroglobulin, DNA, mitochondria, and microsomes, is relatively common, especially in older individuals and does not always lead to overt pathological changes. However, certain autoantibodies, particularly those directed against surface components of such cells as erythrocytes, frequently produce severe autoimmune disease. Other autoimmune diseases, such as Hashimoto's disease (a chronic and progressive thyroiditis), pernicious anaemia, and adrenalitis, are accompanied by infiltrations of mononuclear cells, and cell-mediated responses are believed to contribute to these pathological manifestations.

The role of viruses in the etiology or pathogenesis of autoimmune disease has long been suspected (Lindenmann & Klein, 1967). When considering this problem, however, one must distinguish between immune reactions directed against virus-specified antigens and those directed against host antigens (true autoantigens). Such distinctions may be difficult to make in practice, especially where viruses are transmitted from mother to offspring and are present throughout life. Furthermore, while a virus can often be proved to be present, formal proof of its absence is far more difficult to obtain.

---

[1] Further information on some diseases of animals in which autoimmune manifestations may be related directly or indirectly to an underlying virus infection (in some cases surmised but not proved) is given in Annex 1.

Theoretically, virus infections could precipitate autoimmune reactions in a variety of ways: (1) The virus itself might provide antigens (e.g., viral nucleoprotein) cross-reacting with host antigens; (2) viruses might unmask or release antigens from damaged cells—autoantibodies directed against soluble nuclear components have been found, for example, after infectious mononucleosis; (3) viruses might alter host-cell antigens and act as " helper determinants " (Allison et al., 1971); (4) viruses might derepress host-cell antigens (e.g., embryonic antigens); and (5) viruses might affect the proliferation or responses of immunocompetent cells or their precursors (see *Bull. Wld Hlth Org.*, 1972).

For many years it has been speculated that myxoviruses may cause autoimmune disease. These viruses contain neuraminidase, an enzyme known to produce antigenic modification in host cells. Incubation of erythrocytes with certain myxoviruses results in the unmasking of T-agglutinins, and immunization of animals with the altered red cells can raise serum titres of antibody to these antigens. In addition, elevated T-agglutinin levels have been observed following natural myxovirus infection, but no obvious autoimmune manifestations have been reported. Thus, the relationship between myxovirus infection and autoimmune disease remains unclear.

It has been shown that animals recovered from tumours destroyed by lysis after infection with non-oncogenic viruses (Koprowski et al., 1957), or immunized with homogenates (Lindenmann & Klein, 1967) are more resistant to subsequent challenge with viable uninfected tumour cells than are animals immunized with uninfected tumour homogenates. Several investigators have postulated that the virus modifies the surface antigens of the tumour by introducing a " helper determinant " (Hirsch et al., 1968). These helper determinants would act in much the same way as a hapten to increase the host's ability to mount an effective immune response and reject the tumour. Similarly, it has been suggested that viruses acting as helper determinants might initiate an autoimmune response to antigens of the host's own cells. An alternative hypothesis is that host antigens released from virus-infected cells or host antigens incorporated into the envelope of the maturing virion might reach immunologically competent cells and stimulate the production of specific antibody. These hypotheses have been proposed to explain a number of diseases, including subacute thyroiditis and postinfection encephalitis, but firm experimental evidence is still lacking.

Antibodies to normal cell constituents have been reported in a variety of other diseases known or suspected to be caused by viruses. Antinuclear antibodies (ANA) have been observed in Aleutian disease of mink, equine infectious anaemia (Henson et al., 1970; Squire, 1968), systemic lupus erythematosus (SLE) in dogs and man (Lewis & Schwartz, 1971; Blomjous & Feltkamp-Vroom, 1971), hepatitis in man (Zuckerman, 1971), as well as in New Zealand Black (NZB) mice (Blomjous & Feltkamp-Vroom, 1971). Antibodies to erythrocytes have been reported in Aleutian disease, SLE, NZB mice and EIA (Squire, 1968). Antibodies to mitochondria and smooth muscle have been found in hepatitis, and antibody to thyroglobulin has been detected in dogs with SLE. In some of these conditions—e.g., SLE, a viral etiology has not been established, while in others it is still far from clear whether the autoimmune manifestations are a direct or an indirect consequence of the virus infection. Even in the widely studied NZB syndrome, many questions still remain concerning the relationship between the C-type particles observed in the tissue of these animals and the rise in antibody to erythrocytes and nucleic acids (East, 1970).

Recently, several electron microscopy studies have detected tubular structures resembling the nucleocapsids of myxoviruses or paramyxoviruses in the glomeruli and synovia of patients with SLE (Melczer et al., 1962; Györky et al., 1969; Sinkovics, 1971), in the skin of patients with scleroderma and dermatomyositis (Norton et al., 1970). and in the thymus of patients with myasthenia gravis and Hashimoto's disease (Loghem, 1965). Although these diseases are believed to have an autoimmune component, it is not known how their pathogenesis may be related to the virus-like particles.

Recommendations

(1) A clear distinction should be made between immune responses to viral antigens (which should not be considered under the topic of autoimmunity) and immune responses to host or modified host antigens.

(2) Assay methods should be developed and standardized for the detection and quantification of both the humoral and the cellular immune response to host antigens.

(3) The ability of a virus to cause autoimmune disease should be determined by inoculating animals with a cell-free preparation of purified virions.

(4) In diseases of unknown etiology where auto-immune manifestations have been demonstrated or are strongly suspected, a search for a viral etiology should be initiated. In humans, SLE, rheumatoid arthritis and subacute thyroiditis appear to be good candidates for study (see below). In animals, canine (Lewis & Schwartz, 1971) and feline SLE (Slauson et al., 1971) should receive further attention.

GENETICS

Genetic differences in the susceptibility of animals to virus infections have been well established. Genetic factors linked to the major histocompatibility loci of the mouse may determine the susceptibility of the animals to infection with, e.g., LCMV and some of the oncogenic viruses (WHO Scientific Group on the Genetics of the Immune Response, 1968). When mice of the H-2^k haplotype are exposed to oncogenic viruses such as the Gross (WHO Scientific Group on the Genetics of the Immune Response, 1968), Friend, Rauscher, or Moloney virus, they develop leukaemia, whereas mice of the H-2^b haplotype show much less sensitivity to these viruses. Susceptibility to various types of RNA avian leucosis virus is also genetically determined (WHO Scientific Group on the Genetics of the Immune Response, 1968), and a single dominant factor seems to account for the resistance of one strain of mice to influenza virus (WHO Scientific Group on the Genetics of the Immune Response, 1968). A single gene also is responsible for the resistance of mice to group B arbo-virus infections (Hanson & Koprowski, 1969).

That there are genetic differences in the immune response of the host to a variety of nonviral antigens has also been well documented. In some cases, the magnitude of the immune responses to defined antigens is under the control of allelic genes that are closely linked to major histocompatibility genes. It is possible that the nature and magnitude of the immune response to viral antigens may likewise be under genetic control and may affect immunopathological manifestations. Although genetic control has not been documented with viral antigens, certain animal experiments are consistent with this possibility. The association of the IR-1 gene with the H-2^k haplotype may indicate that "genetic resistance" to oncogenic viruses is expressed by the magnitude of the immune response of the animal host (*Fed. Proc.*, 1972). Similarly, the genetic resistance of C57BL mice to ectromelia virus is thought to be reflected by their greater immunologic reactivity to

the virus (Schell, 1960). Conversely, the weaker immune response of C57BL mice to LCMV is believed to be responsible for the less severe LCMV-induced immunopathological lesions in this strain (Tosolini & Mims, 1971; Mims & Tosolini, 1969).

In man, there is some evidence that differences in the immune response may be a factor in susceptibility to hepatitis (Blumberg et al., 1969; Ceppellini et al., 1970), Indian childhood cirrhosis (Nyak et al., 1972), and subacute sclerosing panencephalitis (SSPE) (Lischner et al., 1972), but it is not certain whether the depressed immune response is genetically determined and precedes the infection or whether it is a result of the infection.

Recommendations

(1) In view of the correlation in mice between H-2 type and susceptibility to RNA virus and the analogy between H-2 and human histocompatibility antigens (HL-A), studies are now in progress to search for an association between HL-A phenotype and susceptibility to diseases with a suspected viral etiology, such as Hodgkin's disease and acute leukaemia. The preliminary studies on SLE should be continued and extended to include rheumatoid arthritis, myasthenia gravis, and eventually other autoimmune diseases of possible viral etiology.

(2) Tests for cell-mediated immunity are not well standardized, as shown by the conflicting reports not only on SSPE (Lischner et al., 1972), but also Hodgkin's disease and other conditions. It is recommended that tests be carried out under uniform conditions, using internationally standardized reagents (*Bull. Wld Hlth Org.*, 1971). The World Health Organization may be able to facilitate the establishment and dissemination of standard tests for cell-mediated immunity. Such tests should be undertaken, for instance, in regions where the incidence of Indian childhood cirrhosis is high (see above).

(3) Studies to investigate possible linkage relationships between hereditary diseases and genetic markers present in blood cells and serum proteins should be encouraged.

IMPLICATIONS FOR CLINICAL RESEARCH

Examples of each of the principal virus-associated immunopathological mechanisms discussed in Parts 1 and 2 of this memorandum are known in experimental animals and provide an opportunity for detailed

analysis of the factors involved. While it would not be possible to conduct similar research on human subjects, the knowledge acquired in animal studies may be applicable to human disease.

In certain virus infections of man, immunopathological complications are suspected of contributing to the disease syndrome. These infections include hepatitis, virus-related haemorrhagic fevers (dengue almost certainly, and perhaps Argentinian haemorrhagic fever), respiratory syncytial virus infection, infectious mononucleosis, and possibly SSPE. Moreover, immunopathological mechanisms may be involved in causing the skin lesions—e.g., rashes—associated with some of the common virus infections of man, such as measles and vaccinia.

Conversely, there are some recognized immunopathological diseases in which viruses are suspected of playing a role. For instance, in immune-complex glomerulonephritis of man the antigen may ultimately prove to be viral in origin. Virus-like particles have been found in patients with SLE (as well as in a dog with SLE); these may be involved in the pathogenesis of the disease syndrome. More extensive investigations are needed to clarify the role of viruses in the etiology of these diseases. Viruses have also been suspected in the etiology of rheumatoid arthritis, but thus far no proof of this has been obtained. The chronic arthritis associated with chlamydial disease in animals and with Reiter's disease in man may have immunopathological components but it is unlikely that these diseases are useful models for rheumatoid arthritis. A possible viral etiology involving immunopathological mechanisms has also been suggested for endocrine diseases, e.g., subacute thyroiditis, and haemolytic anaemia.

In conclusion, the theoretical concepts and technical methods summarized in this memorandum may be usefully applied to the study of suspected immunopathological manifestations in human disease, including autoimmune reactions. The experimental finding that a disease can be prevented or attenuated by the use of immunosuppressive drugs strongly implies that it has an immunopathological component. However, caution should be exercised in attempting to use immunosuppression in human patients, because of the risk of increasing the ability of the virus to replicate and thereby disseminate the infection.

* * *

A. C. ALLISON, Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex, England

W. I. B. BEVERIDGE, Veterinary Public Health Consultant, World Health Organization, Geneva, Switzerland

W. C. COCKBURN, Chief Medical Officer, Virus Diseases, World Health Organization, Geneva, Switzerland

JUNE EAST, Department of Environmental Carcinogenesis, Imperial Cancer Research Fund, Mill Hill, London NW7 18D, England

H. C. GOODMAN, Chief Medical Officer, Immunology, World Health Organization, Geneva, Switzerland

H. KOPROWSKI, The Wistar Institute of Anatomy and Biology, Philadelphia, Pa., USA

P.-H. LAMBERT, World Health Organization Haematology Research Unit, Cantonal Hospital, Geneva, Switzerland

J. J. VAN LOGHEM, Department of Immunopathology, University of Amsterdam, Netherlands

P. A. MIESCHER, Haematology Division, Cantonal Hospital, Geneva, Switzerland

C. A. MIMMS, Microbiology Department, The John Curtin School of Medical Research, Australian National University, Canberra City, Australia

A. L. NOTKINS, Chief, Virology Section, National Institute of Dental Research, Bethesda, Md., USA

G. TORRIGIANI, Medical Officer, Immunology, World Health Organization, Geneva, Switzerland

RÉSUMÉ

ÉTATS IMMUNOPATHOLOGIQUES INDUITS PAR LES VIRUS: MODÈLES ANIMAUX ET RELATIONS AVEC LES MALADIES HUMAINES: 2. IMMUNITÉ À SUPPORT CELLULAIRE, MALADIES AUTO-IMMUNES, GÉNÉTIQUE ET CONSÉQUENCES POUR LA RECHERCHE CLINIQUE

Dans cette deuxième partie du mémorandum, on décrit d'autres mécanismes par lesquels l'interaction d'un virus et du système immunitaire de l'hôte est susceptible de provoquer des lésions tissulaires.

L'immunité à support cellulaire joue un rôle vital dans la résistance aux infections virales et dans le processus de guérison mais, dans certains cas, la réaction entre les cellules immunes et les antigènes viraux, libres ou fixés à la surface des cellules infectées, peut léser gravement les tissus et même entraîner la mort. L'infection de souris (pendant la période néonatale ou, à l'état adulte, après administration de médicaments immunosuppresseurs) par le virus de la chorioméningite lymphocytaire détermine une invasion massive des cellules par le virus, mais les animaux ne présentent que très peu de symptômes cliniques de maladie pendant de longues

périodes. Si on leur administre ensuite par transfusion des cellules immunes, les lésions tissulaires apparaissent et la mort survient, la réaction étant similaire à celle qui succède à l'infection de la souris adulte normale par le même virus.

On pressent depuis longtemps que les virus jouent un rôle dans l'étiologie ou la pathogénie de certaines maladies auto-immunes, et les résultats de recherches récentes plaident en faveur de cette hypothèse. On a signalé l'apparition d'anticorps dirigés contre les constituants normaux de la cellule au cours de plusieurs infections virales. On admet, comme explications les plus plausibles, que a) certains antigènes viraux seraient semblables aux antigènes de l'hôte; b) les virus pourraient révéler la présence d'antigènes cellulaires ou les libérer; c) les virus pourraient modifier les antigènes cellulaires de l'hôte et/ou se comporter comme des « déterminants accessoires ». La microscopie électronique a récemment détecté des structures de type viral dans les tissus d'ani-maux et de malades atteints de certaines affections à composante immunologique, comme le lupus érythémateux disséminé et la maladie de Hashimoto.

Les différences génétiques entre individus, au sein d'une espèce, peuvent influencer les manifestations immunopathologiques dues aux virus. D'après certaines observations, il apparaît que les facteurs génétiques contrôlent non seulement la réceptivité à l'infection virale — la réceptivité à l'infection par les virus de la leucémie, par exemple, est liée à certains antigènes héréditaires d'histocompatibilité — mais encore qu'ils conditionnent en partie l'aptitude de l'hôte à édifier une réponse immunitaire à l'égard des virus et d'autres antigènes.

Le mémorandum formule des recommandations relatives aux investigations futures dans le domaine de l'immunopathologie chez l'homme et chez des modèles animaux et suggère différentes orientations pour la recherche. Certaines affections humaines et animales à composante immunologique sont brièvement évoquées.

REFERENCES

Allison, A. C. (1972) *Immunity against viruses.* In: *The scientific basis of medicine, annual review for 1972,* London, Athlone Press, p. 49

Allison, A. C. et al. (1971) Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity, *Lancet,* **2,** 135

Aoki, T. et al. (1970) G (Gross) and H-2 cell surface antigens: location on gross leukaemia cells by electron microscopy with visually labelled antibody. *Proc. Nat. Acad. Sci. (Wash.),* **65,** 569

Blanden, R. V. (1971 Mechanisms of recovery from a generalized viral infection: mousepox. II. Passive transfer of recovery mechanisms with immune lymphoid cells, *J. exp. Med.,* **133,** 1074

Blomjous, F. J. E. M. & Feltkamp-Vroom, T. M. (1971) Hidden antinuclear antibodies in seronegative SLE patients and in NZB and NZB×NZW F₁ mice, *Europ. J. Immunol.,* **1,** 396

Blumberg, B. S. et al. (1969) Hepatitis and Australia antigen: autosomal recessive inheritance of susceptibility to infection in humans, *Proc. Nat. Acad. Sci. (Wash.),* **62,** 1108

Bull. Wld Hlth Org., 1971, Primary immunodeficiencies, **45,** 125

Ceppellini, R. et al. (1970) *High frequency and family clustering of an antigen in some Italian populations.* In: *Australia ed epatite antigene virale,* Turin, Minerva Medica

Cole, G. A. et al. (1972) Lymphocytic choriomeningitis virus: pathogenesis of acute central nervous system disease (in press)

East, J. (1970) Immunopathology and neoplasms in New Zealand Black (NZB) and SJL/J mice, *Progr. exp. Tumor Res. (Basel),* **13,** 84

Fed. Proc., 1972, Biological significance of histocompatibility antigens: report of an NIH–WHO Conference, **31,** 1087

Glasgow, L. A. (1970) Cellular immunity in host resistance to viral infections, *Arch. int. Med.,* **126,** 125

Györkey, F. et al. (1969) Systemic lupus erythematosus and myxovirus, *New Engl. J. Med.,* **280,** 333

Hanson, B. & Koprowski, H. (1969) Interferon mediated natural resistance of mice to arbo B virus infection *Microbios,* **1B,** 51

Hellström, K. E. & Hellström, I. (1970) Immunological enhancement as studied by cell culture techniques, *Ann. Rev. Microbiol.,* **24,** 343

Henson, J. B. et al. (1970) *Recent research on the virology, serology and pathology of equine infectious anaemia.* In: *Proceedings of the 2nd International Congress on Equine Infectious Diseases, Paris,* Basle, Karger, p. 178

Koprowski, H. et al. (1957) Enhancement of susceptibility to viruses in neoplastic tissues, *Texas Rep. Biol. Med.,* **15,** 559

Lewis, R. M. & Schwartz, R. S. (1971) Canine systemic lupus erythematosus: genetic analysis of an established breeding colony, *J. exp. Med.,* **134,** 417

Lindenmann, J. & Klein, P. A. (1967) Viral oncolysis: increased immunogenicity of host cell antigen associated with influenza virus, *J. exp. Med.,* **126,** 93

Lischner, H. W. et al. (1972) *New Engl. J. Med.,* **286,** 786

Loghem, J. J. van (1965) Viral infection and idiopathic autoimmune diseases, a hypothesis, *Vox. Sang. (Basel),* **10,** 1

Lundstedt, C. (1969) Interaction between antigenically different cells: virus-induced cytotoxicity by immune lymphoid cells *in vitro, Acta. path. microbiol. scand.,* **75,** 139

Melczer, M. et al. (1962) Data on the etiology of lupus erythematosus, *Orv. Hetil*, **103**, 581-584

Mims, C. A. & Tosolini, F. A. (1969) Pathogenesis of lesions in lymphoid tissues of mice infected with lymphocytic choriomeningitis (LCM) virus, *Brit. J. exp. Path.*, **50**, 584

Norton, W. L. et al. (1970) Endothelial inclusions in dermatomyositis, *Ann. rheum. Dis.*, **29**, 67

Nyak, N. C. et al. (1972) α-Fetoprotein in Indian childhood cirrhosis, *Lancet*, **1**, 68-69

Oldstone, M. B. A. & Dixon, F. J. (1970a) Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. II. Relationship of the anti-lymphocytic choriomeningitis immune response to tissue injury in chronic lymphocytic choriomeningitis disease, *J. exp. Med.*, **131**, 1

Oldstone, M. B. A. & Dixon, F. J. (1970b) Tissue injury in lymphocytic choriomeningitis viral infection: virus-induced immunologically specific release of a cytotoxic factor from immune lymphoid cells, *Virology*, **42**, 805

Oldstone, M. B. A. & Dixon, F. J. (1971) *The immune response in lymphocytic choriomeningitis viral infection.* In: P. A. Miescher, ed., *Immunopathology: VIth International Symposium, 1970*, Basel, Schwabe, p. 391

Schell, K. (1960) Studies on the innate resistance of mice to infection with mousepox. I. Resistance and antibody production, *Aust. J. exp. Biol. med. Sci.*, **38**, 271

Sinkovics, J. G. (1971) Virus-like particles in systemic lupus erythematosus, *New Engl. J. Med.*, **284**, 107

Slauson, D. O. et al. (1971) Naturally occurring immune complex glomerulonephritis in the cat, *J. Path. Bact.*, **103**, 131

Speel, L. F. et al. (1968) An immuno-cytopathogenic interaction between sensitized leukocytes and epithelial cells carrying a persistent noncytocidal myxovirus infection, *J. Immunol.*, **101**, 409

Squire, R. A. (1968) Equine infectious anaemia: a model of immunoproliferative disease, *Blood*, **32**, 157

Tompkins, W. A. F. et al. (1970) An *in vitro* measure of cellular immunity to fibroma virus, *J. Immunol.*, **104**, 502

Tosolini, F. A. & Mims, C. A. (1971) Effect of murine strain and viral strain on the pathogenesis of lymphocytic choriomeningitis infection and a study of footpad responses, *J. infect. Dis.*, **123**, 134

WHO Scientific Group on Cell-Mediated Immune Responses (1969) *Wld Hlth Org. techn. Rep. Ser.*, No. 423

WHO Scientific Group on the Genetics of the Immune Response (1968) *Wld Hlth Org. techn. Rep. Ser.*, No. 402

Zuckerman, A. J. (1971) *The immunopathology of viral hepatitis associated with Australia antigen.* In: P. A. Miescher, ed., *Immunopathology: VIth International Symposium*, Basel, Schwabe, p. 436

Annex 1

Murine autoimmune haemolytic anaemia

Haemolytic disease begins to develop in some mice of the NZB strain at the age of 4-5 months; direct Coombs reactions increase in incidence and intensity until the majority of animals are positive at age one year. These serologic reactions are accompanied by anaemia of insidious onset and variable degree and by progressive reticulocytosis and splenomegaly. The circulating autoantibody reacts with the erythrocytes of normal strains of mice in the indirect Coombs test. Impairing the cell-mediated immune responses by neonatal thymectomy neither delays nor prevents Coombs conversion, which shows that the reaction is antibody-mediated. Persistent high levels of IgM are found.

C-type viruses, very similar to known viruses of the murine leukaemia group, are regularly observed in conventional and germ-free NZB embryos and in animals of the NZB strain throughout life. Presumably, the virus is transmitted via the germ-cells or placenta, or both, in the same way as the indigenous Gross virus of other spontaneously " highly leukaemic strains ". The NZB virus possesses antigens of the Gross type. The fact that germ-free NZB mice also develop autoimmune haemolytic anaemia suggests that the stimulus precipitating autoimmunity is intrinsic and might be related to the animals' own endogenous murine leukaemia virus. There is still no convincing experimental evidence that the virus in the form of cell-free filtrates can reproduce the autoimmune disease in either syngeneic or allogeneic recipients.

Male or female NZBs can, nevertheless, transmit the disease to their hybrid offspring when mated with normal partners, and since the transmission patterns cannot be easily interpreted in simple genetic terms a viral etiology must still be suspected. Coombs reactions do not develop in other highly leukaemic strains of mice. This implies that the NZBs react differently to their Gross leukaemia virus, as indicated by the observation that they produce cytotoxic and possibly virus-neutralizing antibody. If their thymocytes are sensitized to viral antigens, a helper

effect may contribute to autoimmunity. However, on the basis of present evidence, it is still a matter for conjecture whether the virus contributes to the development of autoimmunity in NZB mice.

Murine SLE

Moderate to severe membranous glomerulo-nephritis with proteinuria develops in both germ-free and conventional NZB mice as they age, but only a few experience severe histologic damage and renal failure. However the lesions of F_1 (NZB× New Zealand White [NZW]) hybrids are more severe, the majority of F_1 females dying within a year. The hybrids spontaneously develop high titres of circulating anti-DNA, which together with DNA and complement accumulates in the mesangium and glomerular capillaries. Antibodies to RNA also circulate in young animals. Immunization of very young hybrids with single stranded DNA can hasten the appearance of ANA and cause a fatal acceleration of the glomerulonephritis. Synthetic double-stranded RNA without adjuvant provokes the formation of anti-RNA and/or anti-DNA complexes and, with adjuvant, exacerbates the kidney disease and forms complexes in the kidneys.

Other strains spontaneously develop ANA, some in very high incidence, and are able to produce specific ANA when suitably immunized. The titres of ANA obtained vary with the strain, but high titres are sometimes seen. The severe kidney disease of the NBZ×NZW F_1 hybrids is only one aspect of their intrinsic hyperreactivity to some specific antigenic stimuli, including DNA. A contribution by the endogenous Gross virus is suggested by the fact that glomerulonephritis still develops in germ-free NZB mice, in which type-specific viral antigen and anti-viral antibody are also present in the glomerular deposits. The most likely explanation is that the Gross virus triggers or reinforces the autoimmune reaction, resulting in glomerulonephritis. However, the problem has yet to be solved, and the source of the DNA is still uncertain. In view of the presence of an RNA-dependent DNA polymerase in C-type particles, it is conceivable that the RNA in the virions may be transcribed into DNA proviruses, thus providing material which either induces or reacts with ANA.

There is increasing evidence that two factors are involved in the pathogenesis of the lupus type of glomerulonephritis in mice. Mice carrying a large amount of Gross virus develop anti-DNA antibody and a lupus-type glomerulonephritis, provided they have a good immune reactivity to DNA-like antigens. In contrast, in NZB×NZW F_1 hybrids infected with lactic dehydrogenase virus, depression of natural Gross virus infection leads to an inhibition of the renal disease.

Human SLE

The distinctive features of human SLE are the anti-DNA antibodies and the immune complexes containing DNA present in the kidneys. Tubular structures resembling the nucleocapsids of myxo- or paramyxoviruses have frequently been observed by electron microscopy in the endothelial cells of renal glomeruli and, less often, in the lymphocytes and synovia of patients with SLE. Similar particles have been found in skin biopsies of patients with scleroderma and dermatomyositis, in the thymus of patients with myasthenia gravis or Hashimoto's disease, and in the stomach of one person with pernicious anaemia. The particles are situated in the cytoplasm of epithelial and endothelial cells and fibroblasts; they may be localized around the nucleus or lie free in the dilated cisternae of the endoplasmic reticulum. There is evidence that they contain RNA.

Canine SLE

A canine counterpart of human SLE has been described, characterized by LE cells, circulating ANA, and fatal glomerulonephritis, associated with a severe Coombs-positive haemolytic anaemia often accompanied by thrombocytopenic purpura. Polyarthritis, antibody to thyroglobulin, rheumatoid factor, and hyperglobulinaemia are also found. A breeding colony of affected dogs has now been established, and the offspring of affected parents, or an affected mother, exhibit multiple serologic abnormalities and thymic lesions but no clinical signs as yet of SLE. The data obtained to date do not point to a simple pattern of genetic inheritance, and the possibility of a vertically transmitted infectious agent has to be considered. One dog examined has had virus-like particles in renal glomerular endothelium similar to those reported in human SLE. An SLE-like condition has also been observed in cats.

Equine infectious anaemia

This is a disease produced by a virus transmitted mechanically by insects and characterized by vascular lesions, anaemia, and glomerulonephritis. Splenomegaly and lymphadenopathy are accompanied by

proliferation of atypical plasma cells; serum IgM increases concomitantly with clinical episodes but high titres may persist in chronic cases. The virus, growing in cultured lymphocytes, is very similar although not identical in ultrastructure to C-type particles of leukaemia viruses. The infected horses are C3-deficient, and complement coats the erythrocytes of some animals at various stages of the disease. It is possible that virus antibody-complexes on the surface of the erythrocytes account for the presence of complement, in which case there is no need to postulate the presence of true autoimmunity. This condition would then be considered among the immune-complex diseases.

Mycoplasma infection

Mycoplasma pneumoniae infection in man is regularly followed by the transient appearance of cold autoantibody with specificity against the I or i blood group antigen. It has also been reported that antibodies to some cell components of lung tissue are present, and this finding would be worth confirming. Antibodies with anti-I specificity have been reproduced in one experiment by inoculating M. pneumoniae into rabbits. Claims that rheumatoid arthritis is related to infection by M. fermentans have not been substantiated, and there is as yet no strong evidence for a viral etiology of this disease.