



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

## VIRUS-RELATED PATHOLOGY: IS THE CONTINUED PRESENCE OF THE VIRUS NECESSARY?

J. Huppert and T. F. Wild

Unité de Virologie Fondamentale et Appliquée  
I.N.S.E.R.M.—Unité 51  
Unité Associée No. 613 du C.N.R.S.  
69371 Lyon Cedex 08, France

### I. THE CONCEPT

Is the presence of virus necessary for all pathological manifestations related to virus infection? We were confronted by this question after a decade of research on what was termed, according to Fenner, "slow virus infections." Progressively, we evolved toward the idea of virus infections with a delayed pathology. The main effort during this period was to study how, and in which form, virus information persisted in cells and in the organism. More recently, in view of the results from our group and from the literature, we began to evaluate critically whether the continuing presence of the virus was always necessary for the appearance of the pathological phenomenon. Last year we published an article with the provocative title, "Virus Disease without Virus," to present the concept that viruses may play a triggering role for several clinical manifestations, even after the virus has been eliminated (Huppert and Wild, 1984).

What mechanisms can be involved in such situations? Viruses can provoke chromosome modifications. If cell death does not ensue, the alterations may affect the regulation of gene expression. Repair of chromosomal translocation is a infrequent event. By analogy to this situation, the continuing presence of chemical mutagens or carcinogens is not necessary for the transformed state. Once the agent has produced its effect on the cellular genome, its phenotypic manifestation will depend on the altered expression. Such mutations were studied to elucidate "carcinogenesis" rather than to define metabolic modifications.

A second mechanism of virus-induced late pathology concerns the immune system. Viruses are excellent immunogens and stimulate both the humoral and cellular responses. They can also infect the cells of the immune system, and disturb their normal functioning. Finally,

in recent years, it has been shown that interferon may be the origin of a number of pathologies. Viruses are, of course, one of the best inducers of interferon.

When we started to prepare this article, the task was made difficult, as in all new concepts, since the established key words in the literature did not correspond to the phenomenon we wished to describe. The nearest formulations were (1) "hit and run" and (2) "luxury function." (1) In 1983, Galloway and McDougall proposed the term "hit and run" mechanism. Transfection of cells with herpes virus DNA fragments lead to transformation, but the viral DNA could no longer be detected. (2) Oldstone *et al.* (1982) introduced the concept that viruses may alter nonvital luxury functions, such as hormone synthesis, of differentiated cells. This in turn may produce an indirect effect on a different organ.

Despite the limitations of the key word system, a search through the literature for the mechanisms we have cited revealed a wealth of information which strongly reinforced our feeling that virus disease without virus really exists. We apologize in advance to many authors who have published observations concerning this field and who have not been cited. We hope that this review and our concept will stimulate further fruitful research.

## II. SOME GENERAL CONSIDERATIONS

Although certain viruses have a potential to cause a number of pathological conditions, the outcome depends on a number of factors. The primary site of replication for a virus is determined by the route of entry into the host. During the viremia which follows, virus is disseminated to other tissues. At this point, the immunological system may intervene limiting the further spread of virus. During the viremia, a number of cell types will come in contact with the virus, but the ability to infect the cells will depend upon the tropism of the virus. Thus, viruses multiplying in lymphatic cells (measles) or adhering to these cells (reovirus) may have the possibility to pass into the brain.

The role of the immunological system is initially to neutralize the extracellular virus, preventing further dissemination, and secondarily to eliminate the infected cells. If the infected cell is easily replaced, such as the epithelial cells of the intestine, then a lytic infection in such cells will not have a long-term pathological effect on the host. If, however, the virus destroys cells which cannot be replaced, then the infection will impose a permanent direct pathology on the host.

An example of such infections is observed with poliovirus. This virus

normally replicates in the cells of the gastrointestinal tract. The majority of infections are asymptomatic, but in certain cases the virus may generalize and pass into the nervous tissue. Here, the virus can infect neurons which will not be replaced. Depending on the involvement of the nervous tissue, a state of paralysis may ensue. The direct loss of irreplaceable nervous tissue is responsible for the pathological lesion. This persists after the virus has been eliminated.

Certain other virus infections do not fall into the above categories. They have either adapted their replication to avoid immunological elimination, or their multiplication is modified by a combination of events. In these systems, the virus may remain in the host cells, either latently or as a persistent infection. In the case of RNA viruses, it has been shown that the less selective environment of a nonlytic system permits the accumulation of mutations in the virus which are normally lost in the lytic system (Holland *et al.*, 1982). During the evolution of the infection these mutations may give rise to proteins which may be rapidly degraded or can no longer function biologically.

Such mutations observed *in vivo* lead to defective viruses. Further evolution within such cultures may lead to even greater reductions in the expression of the virus genes, a situation which has been ascribed to measles virus in subacute sclerosing panencephalitis (Haase *et al.*, 1985). Despite this diminishing virus expression, the clinical state continues to deteriorate.

Following this line of argument to its end, is it possible for the virus to become so defective that it is eventually lost to the system? It has been shown that cells persistently infected by measles can lose their virus after cell cloning (Wild *et al.*, 1981) and reovirus can be removed from persistently infected cultures by treatment with virus-specific antibody. Whether this situation can arise *in vivo* has yet to be demonstrated. If a virus is eliminated from a cell, does it permanently modify any of the normal cellular processes, and if so, do they contribute to a pathological condition?

During the past few years, a number of animal models have been developed in which viruses induce endocrine disorders. Some of these diseases have been directly correlated to a cell killing effect on the hormone-producing cells. Others have been linked with the presence of persistent infections in the endocrine cell whereas a further type triggers an autoimmune disease in which antibodies against various tissues and hormones are produced. Thus a virus that limits the availability of hormones may provoke a metabolic disorder. In each of these cases, is the continued presence of the virus necessary for the manifestation of the disease? The questions raised by the above observations have wide-ranging consequences in human medicine.

## III. VIRUSES AND CHROMOSOMES

A. *Virus-Induced Cytogenic Changes*

The development of human cytogenetics, around 1960, demonstrated that certain human diseases may be associated with chromosome anomalies. These studies were rapidly extended to animal models and cells in culture. It was soon realized that viruses had the potential to induce chromosome anomalies. In 1961, Hampar and Ellison were the first to describe chromosome modifications in hamster cells infected with herpesvirus. In 1962, Nichols observed chromosome pulverization in lymphocytes from peripheral blood of patients with acute measles. Koprowski *et al.* (1962) and Shein and Enders (1962) transformed human cell cultures with SV40 virus and observed modified karyotypes. Landa *et al.* (1962) described the modified karyotype of rat tumors (XC) provoked by Rous sarcoma virus. By that time, epidemiological data provided evidence that rubella in early pregnancy is responsible for severe fetal malformations. This was confirmed by Boué *et al.* (1964), who showed that *in vitro* infection of skin or pharyngeal mucosa explants of human embryo with rubella virus provoked chromosome breaks in 43% of the analyzed metaphase cells.

Such studies were rapidly expanded to most known animal viruses. Already in 1970, Nichols and also Stich and Yohn, in excellent general reviews, could cite the following groups of viruses as possessing the capacity to induce chromosome alterations in cultured cells: papovaviruses: SV40; adenoviruses: human ADV 2,4,7,12, and 18, and simian ADV 7 and 15; herpes group: herpes simplex type 1 and 2, varicella-zoster; poxviruses: vaccinia; picornaviruses: poliomyelitis; myxo- (para- and ortho-) viruses: mumps, Newcastle disease (NDV), Sendai, measles, rubella, influenza; retroviruses: Rous sarcoma.

They also listed virus infections which were shown to be accompanied by chromosome aberrations in patients' circulating lymphocytes: hepatitis, meningitis, chicken pox, mononucleosis, fourth diseases, mesenteric lymphadenitis, mumps, and measles.

Later the list was extended to Epstein-Barr virus (Huang, 1971), tick-borne encephalitis (Ilinskikh and Ilinskich, 1976), rabies (Majer *et al.*, 1977), Venezuelan encephalitis (Pruslin and Rodman, 1978), and cytomegalovirus (Luleci *et al.*, 1980). Apparently whenever karyological studies were performed on virus-infected cells or patients' lymphocytes from blood or bone marrow, chromosome alterations were found. The only published exception was in Tacaribe virus-infected cells (arenavirus group). Damonte *et al.* (1979) found no difference in the karyological pattern, including G and C banding between normal

Vero cells and a line persistently infected with the virus, even though the cells were resistant to superinfection with other arenaviruses. Attenuated vaccine virus strains were, in general, less damaging to chromosomes than the corresponding wild-type virus (Gorshonova and Mikhaïlova, 1976; Csonka *et al.*, 1975; Majer *et al.*, 1977).

Epidemiological studies revealed a possible relationship between infectious hepatitis in mothers and Down's syndrome (Stoller and Collmann, 1965). Furthermore, several syndromes attributed to an abnormal chromosome complement have implicated infectious agents on epidemiological grounds (Day, 1966; Robinson and Puck, 1967). In a laboratory model of influenza virus in mice, Thadani and Polasa (1979a,b) showed that the virus induces a significant increase in the percentage of chromosome anomalies in spermatogonia and spermatocytes.

The chromosome abnormalities provoked by viruses can be grouped under four main categories: (1) chromosome breaks, (2) chromosome pulverization, (3) chromatid exchange or translocations, and (4) modifications of the mitotic apparatus. The most frequently observed lesions are chromosome breaks and pulverization. These typically appear early in infection, and surviving cells present either a normal karyotype or translocations, but not breaks. However, we should remember that a great part of these studies were done before the chromosome banding techniques were in general use. Therefore, translocations may have been seriously underestimated and restricted to situations where a characteristic "marker" chromosome could be identified. Chromosomal rearrangements are certainly of greater biological significance than breaks or pulverizations. Whereas the latter modifications are lethal, chromosomal rearrangements are not and are permanent. Breaks and gaps precede rearrangements but, as they have to be repaired, this can only occur in cells which retain the ability to synthesize proteins and DNA. In the presence of chemical inhibitors of DNA synthesis, only open chromosome breaks are seen. However, after the removal of the inhibitor, chromosomal rearrangements appear (Nichols and Heneen, 1964).

The fourth way in which chromosomes can be modified involves the alteration of the spindle and the mitotic apparatus. This may lead to syncytia formation or to a change in chromosome number by nondisjunction and formation of extra copies of some chromosomes (Nichols, 1970).

### *B. Mechanisms of Chromosome Alterations*

Several hypotheses have been formulated to explain the mode of action of viruses on chromosomes, but none could be clearly proved

until now. Allison and Paton (1965) proposed the breakdown of lysosomes followed by liberation of enzymes, especially nucleases, to be the main mechanism. In careful experiments, Aula and Nichols (1968) compared infection of measles and polioviruses (the latter producing lysosomal breakdown but few chromosome changes). They followed lysosome integrity by measuring the release of acid phosphatase. No correlation was found between the amount of enzyme liberated and chromosome alteration. Furthermore, addition of cortisone, known to stabilize lysosomes, did not prevent chromosome alteration. Sablina (1978) showed that chromosome aberrations of HSV-infected permissive and nonpermissive cells depend on DNases of both lysosomal and virus origin. Most hypotheses generally accept that the inhibition of cellular DNA synthesis by virus infection, or competition for precursors, is a plausible explanation. Hand (1976) showed that in mouse L cells infected with mengovirus or NDV, 5 hours after infection, DNA synthesis was normal despite a marked inhibition of [<sup>3</sup>H]thymidine incorporation. Later, replicative fork progression was slowed down and the number of initiation events decreased. Shelton *et al.* (1981) have found that one of the reovirus nonstructural proteins binds to DNA and may interfere with chromosome function in the infected cell. Bellett *et al.* (1982) reported that actively growing rodent cells infected with human ADV 5 suffer various types of chromosomal damage. ADV uncouples DNA replication and polyamine synthesis. Addition of spermine or aminoguanidine (an inhibitor of diaminoxidase which prevents polyamine catabolism) prevents chromosome damage if added up to 1.25 hours before mitosis. It may be relevant to this that G. A. Quash (personal communication) has found that malondialdehyde (produced by polyamine oxidation) specifically interacts with nucleotides.

The virus effect on chromosomes depends on a number of factors. These include the virus strain, the host cell, and the phase of the cell growth cycle. G<sub>1</sub> being most sensitive. Increasing the multiplicity of infection generally leads to increased chromosome damage. Virus replication, or even infectivity, is not always indispensable for inducing chromosome alterations. In a few cases the activity has been related to a virus-induced protein. With Sendai and measles viruses, the envelope, responsible for cell fusion, is sufficient (Cantell *et al.*, 1966; Norrby *et al.*, 1966). In HSV-infected cells, the synthesis of virus-coded early proteins seems to correlate with chromosome breakage (O'Neill and Rapp, 1971).

In persistently infected cells, the modification of the karyotype is so frequent that it was questioned whether the modified chromosomes were the result of infection or corresponded to the selection of preexist-

ing mutant cells. To answer this question, Homma *et al.* (1968) used HeLa cells persistently infected with type 2 hemadsorption virus (a variant of Sendai) and which have a characteristic karyotypic modification. When HeLa cells were infected with this virus, nearly all the metaphase cells at 40 hours after infection had the modified karyotype characteristic of the persistently infected line. Schwöbel *et al.* (1977) and Epplen *et al.* (1977) studied BHK cell lines persistently infected with Sindbis, NDV, vaccinia, or pseudorabies viruses. Each of these cell lines presented a decreased modal number of chromosomes. R and Q banding revealed specific chromosomal markers for each of the infected lines. A subline of the Sindbis virus persistently infected cells became virus free after 4 years of subculturing. This subline conserved the characteristic modified karyotype. Infection of this cell line with any of the above-mentioned viruses readily gave rise to a persistent infection. Wild *et al.* (1981) found that BGM cells persistently infected with measles virus had a modified karyotype and several biochemical modifications, especially in phospholipids (this is described in another section of this review). Certain cell clones obtained from these cultures no longer contained viruses but they retained their karyotypic and biochemical modifications.

### C. Viruses as Mutagens

The recent studies of cellular *onc* gene expression have clearly demonstrated that chromosomal translocations may activate genes, merely by inserting them in the proximity of a strong initiator or enhancer sequence. Furthermore, it is evident that, in animal cells, transposon-like elements can play an important role in gene expression. Therefore, it would not be surprising if virus-induced chromosome changes could be accompanied by mutations in the cellular properties. However, if the same question has been asked on a number of occasions by investigators, it was mainly from the point of view of transformed cells and their possibility of having acquired tumorigenic properties. Nevertheless, in several reports, modifications of enzymatic activities have been described as a consequence of virus-induced chromosome modification.

Ebina *et al.* (1969) assayed alkaline phosphatase in the HeLa cell line persistently infected with type 2 hemadsorption virus. The amount of enzyme was significantly decreased in comparison to the parental HeLa cells; furthermore, one of the two isozymes existing in HeLa cells practically vanished. Several other enzymes, e.g., glucose-6-phosphate dehydrogenase and other dehydrogenases, remained unchanged. The authors discuss the possibility of this effect



being related to an alteration of chromosome G21, where the alkaline phosphatase gene is located.

SV40-infected nonpermissive hamster cells bear chromosome alterations and present biochemical mutations concerning enzymes such as hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (Marshak *et al.*, 1975), thymidine kinase, and dihydrofolate reductase (Theile *et al.*, 1976). An increased reversion of auxotrophic cell mutants to prototrophy was observed for glutamine (Varshaver *et al.*, 1977) and methionine (Hoffman *et al.*, 1978) after infection with SV40. This virus could induce resistance of cells to drugs such as methotrexate or colchicine, and could even reverse, *in vitro*, the enzymatic deficiency of human Lesch-Nyhans fibroblasts (Theile *et al.*, 1979). Human adenovirus 5 similarly mutated Chinese hamster cells. The mutation rate at the HGPRT locus after virus action was similar to that after treatment by the chemical mutagen ethylmethyl sulfonate (Marengo *et al.*, 1981). The mode of action of herpesviruses is somewhat better understood. They can transform a thymidine kinase-deficient cell by insertion of the virus gene coding for the equivalent enzyme (Munyon *et al.*, 1971, for HSV-1; Yamanishi *et al.*, 1981, for varicella-zoster). HSV is also capable of producing various cell mutations (zur Hausen, 1980; Schlehofer and zur Hausen, 1982). However only virus previously inactivated, generally by UV irradiation, could be used to avoid cell killing. zur Hausen (1980) has proposed that herpesvirus may induce mutations and malignant transformations by activation of cellular repair mechanisms, due to the introduction of damaged foreign DNA. In order to avoid this problem, Pilon *et al.* (1985) used virulent HSV, but with nonpermissive rat XC cells. Under these conditions the mutation rate of the cellular HGPRT locus was still increased by factors ranging between 2.5 and 10.3.

HSV functions are not necessary to maintain the transformed state of cells (in contrast to RNA tumor viruses). This was well established by Galloway and McDougall (1983): mouse 3T3 cells transformed with fragments of HSV DNA conserved the modified characteristics, even in the absence of any virus DNA. These authors considered that malignancy was due to a HSV-induced mutation related to the chromosome modification. They have proposed the term "hit and run" mechanism for this phenomenon.

Several of the papers describing persistently infected cells refer to enzymatic and biochemical changes without giving a detailed study of this aspect of the problem. A very interesting finding, highly relevant to our purpose, was reported by Golubovsky and Plus (1982). *Drosophila melanogaster* stocks contain a contaminating C-picornavirus. A virus-free stock was prepared by outer disinfection of eggs. There

were three times less mutations in the resulting progeny as compared to the isogenic stock with the virus.

#### IV. AUTOIMMUNITY

The immunological repertoire includes antibodies to both foreign and self antigens. Fortunately, in most cases, the antibodies against the cells' own structures are of limited or feeble magnitude and do not lead to a pathological state. However, in certain circumstances, the immunological impetus against certain cellular components can lead to an autoimmune response with pathological consequences. A number of models have been developed to investigate the various parameters of the disease. Here, we will deal with the possible mechanisms by which viruses may influence the autoimmune phenomenon.

Viruses are more than just carriers of antigens. Because of their ability to enter and replicate within cells, viruses challenge the immune system in several ways. Infected cells express virus-specific cell surface antigens that can serve as targets for immune defenses. In addition, viruses may infect macrophages or lymphocytes and affect the nature of the immune response.

Autoantibodies occur at low levels in the sera of normal animals (Karsenti *et al.*, 1977) and humans (Dighiero *et al.*, 1982), but can be markedly elevated during viral infections (Fong *et al.*, 1981; Haire, 1972; Kurki *et al.*, 1978; Lidman *et al.*, 1976; Linder *et al.*, 1979; Toh *et al.*, 1979; Haspel *et al.*, 1983a,b). When the viral infection ceases, the level of the autoantibodies normally falls back to the original levels.

One possible mechanism of importance for this discussion is that, in many cases, it is not virus-induced cell death which induces the pathological manifestations, but rather the triggering of an inappropriate immune response by the virus infection. There are at least three possible mechanisms by which the virus can induce such a state. (1) The virus can act as a general mitogen; (2) the cellular proteins can be modified to render them antigenic; (3) the viruses may share epitopes with cell proteins.

##### A. Polyclonal Lymphocyte Activation

Viruses acting as mitogens can stimulate polyclonal B lymphocyte activation and also enhance nonspecific T lymphocyte helper activity. This, may then activate B lymphocytes previously primed to a wide range of nonviral antigens. Thus, in infections such as chronic hepati-

tis B, high levels of antibodies are found against both unrelated viruses and cellular components (Lidman *et al.*, 1976).

Further evidence that a variety of agents, including viruses, can act as general mitogens to produce autoimmune disease has been provided by a variety of experiments in mice and by observation of patients with autoimmune disease (Theofilopoulos and Dixon, 1982; Tonetti *et al.*, 1970). The effect is observed in both acute and chronic infections in humans and experimental animals (Hirsh and Proffitt, 1975).

A general characteristic of polyclonal B cell activators is that they are often high molecular weight polymers (polysaccharide or protein) containing repeating antigenic determinants. The architecture of a virion fulfills these criteria as, in general, they are constructed of many copies of a few proteins. Viruses which have been shown to be direct mitogens include influenza, vesicular stomatitis, adenovirus, African swine fever, and Sindbis viruses (Butchko *et al.*, 1978; Goodman-Snitkoff and McSharry, 1980a,b; Gibson *et al.*, 1982; Wardley, 1982). In each case, the virus does not need to be infectious.

The actual mechanism of the polyclonal B cell activation is not known. Based on experiments with LCM virus-infected mice, Ahmed and Oldstone (1984) have proposed a possible model. They suggest that the activation is mediated by soluble nonspecific B cell growth and differentiation factors released by the virus-specific helper T cells. Their hypothesis is based on observations that most viruses induce a strong T cell response, resulting in activation of virus-specific helper T cells (Mims, 1982). These factors can induce polyclonal B cells to proliferate and secrete antibody (Dutton, 1975; Altman and Katz, 1982; Muraguchi *et al.*, 1983; Howard and Paul, 1983). The nonspecific B cell growth and differentiation factors have no or little effect on resting B cells, but they can activate cells previously primed with an antigen. At any given time, there are a number of such cells sensitive to the nonspecific lymphokines released by activated T cells. Thus, B cells may be activated indirectly during the infection.

In general, the autoimmune response is transient in acute virus infections. However, in persistent infections, there is a continuous production of autoantibodies, which leads to the formation of antigen-antibody complexes. These may be deposited in the renal glomeruli, arteries, or choroid plexus, thus giving rise to a pathology similar to that observed in interferon-treated mice.

### *B. Modification of Cellular Antigens*

An autoimmune response by polyclonal activation assumes that the host immunological system has already been sensitized to the appro-

priate cellular proteins. For this to occur, it is necessary that the immunological system recognizes certain host cellular proteins as foreign. To do this, during the acute phase the virus infection either modifies certain cellular proteins, or presents them in a modified configuration.

The destruction of cells by viruses and subsequent partial degradation of the cellular products may lead to such autoantibodies. The destruction of oligodendroglia cells by viruses has been shown to have a role in the development of demyelination in experimental animals. However, only circumstantial evidence exists that a virus infection initiates an immune response which contributes to brain injury in infections with canine distemper, Theiler, visna, or vaccinia viruses (Lipton and Dal Canto, 1979; Panitch *et al.*, 1976; Tschannen *et al.*, 1979; Steck *et al.*, 1981). However, Watanabe *et al.* (1983) have shown that in the course of infection of rats with a coronavirus, the lymphocytes were sensitized against basic myelin protein. Adoptive transfer of these lymphocytes after *in vitro* restimulation with the basic myelin protein was followed by experimental allergic encephalitic (EAE)-like lesions in recipient animals. In a further example, in a persistent measles virus infection in hamsters, EAE developed when the animals were challenged with basic myelin protein in combination with Freund's complete adjuvant (Massanari *et al.*, 1979).

Drzeniek and Rott (1969) have proposed that viruses which bud from membranes may lead to membrane modifications, resulting in immunogenic structures. Infection of BGM cells with measles virus exposed certain cellular proteins at the surface (Wild and Greenland, 1979). This phenomenon was also observed in persistently infected cells and was retained even when the cells were cured of virus (Wild *et al.*, 1981). Virus absorption to cell membrane fractions can also render them immunogenic. Eaton (1980) showed that Newcastle disease virus adsorbed to membrane fractions of mouse splenocytes gave rise to complement fixing and cytotoxic antibodies reactive with syngeneic tissue and intact cells. Some mice died from an autoimmune disease.

Thus the mere fusion of the virus envelope with the plasma membrane can orientate cellular proteins to an immunogenic configuration. Taking this one step further, viruses may incorporate cellular proteins into the virions during maturation, and these might evoke an immunological response. The molecular mechanisms controlling the assembly of membrane structures of enveloped viruses are not known. There appears to be an efficient phenotypic mixing of surface glycoproteins between different families of enveloped viruses (Zavada, 1982), but most of the cellular, nonviral surface proteins appear to be efficiently excluded. However, vesicular stomatitis virus (VSV) selectively assem-

bles surface proteins of around 100K from vero cells and from mouse L cells (Lodish and Porter, 1980) and also the Thy-I molecule from mouse lymphoblast cells (Calafat *et al.*, 1983). A number of viruses, including members of the arena-, rhabdo-, and paramyxovirus groups, have been shown to incorporate cellular proteins into the virus particles. Whether these cell proteins become immunogenic under these circumstances is unknown, but measles virus, which incorporates cellular actin into the virion, gives rise to anti-cell antibodies during infections in humans (Haire, 1972).

The presentation of the cellular proteins will also depend upon any changes that may occur in the lipid composition of the plasma membrane during the infection. Although in general the virus does not modify lipid metabolism in acute virus infections, in measles persistent infections fatty acid metabolism can be drastically altered (Anderton *et al.*, 1983). This leads to a change in the composition of the membrane (Anderton *et al.*, 1981), which in turn may influence the presentation of the cell antigens, thus rendering them immunogenic.

Along with other agents, certain viruses possess enzymatic activities such as neuraminidase. These enzymes could be partially responsible for the modification of cellular proteins. Duc Dodon and Quash (1981) have shown that desialylation of rabbit immunoglobulins renders them immunogenic. Repeated inoculations of such desialylated preparations gave rise to rheumatoid factor production and arthritis in rabbits (Galloway *et al.*, 1983). *In vitro*, it has been shown that infection of immunoglobulin-secreting lymphoblast cell lines with an orthomyxovirus leads to the synthesis of a desialylated immunoglobulin (Duc Dodon *et al.*, 1982). Thus, it is possible that infections with viruses containing neuraminidase activities can render the immunoglobulins (or other glycoproteins) immunogenic. Repeated infections by neuraminidase-bearing organisms could possibly lead to an autoimmune state.

### *C. Molecular Mimicry*

With the advent of the hybridoma technique for producing monoclonal antibodies, a large number of banks were established of antibodies against a number of viruses. Among these collections, certain of the antiviral monoclonal antibodies also reacted with cellular proteins. For example, monoclonal antibody to a nonstructural protein of Japanese encephalitis virus bound to a nuclear antigen in uninfected cells (Gould *et al.*, 1983). Monoclonal antibodies to the phosphoproteins of measles and herpesvirus both fixed to the cytoskeletal filaments of

uninfected cells, although the epitopes involved differed (Fujinami *et al.*, 1983). Although an antibody response to a shared antigenic epitope is normally weak and does not result in autoimmunity, it is possible that repeated stimulation by different agents together with the other proposed mechanisms could greatly increase the autoimmune antibody.

Recently, Sheshberadaran and Norrby (1984) have found that certain monoclonal antibodies against the fusion protein of measles virus cross react with the heat shock cellular proteins. Synthesis of these proteins is induced during a number of virus infections. The significance of these observations has to be clarified.

Autoantibodies can potentially be induced by several mechanisms as outlined above. It is assumed that at the end of the infection, except in certain circumstances, these antibodies fall below a detectable level. However, repeated stimulation, either directly, e.g., by desialylation of certain glycoproteins or indirectly as in polyclonal B cell activation, may trigger an autoimmune disease of some consequence. The importance of this phenomenon is that, in each instance, the virus triggers the disease but the continuation of the diseased state takes place in the absence of the virus.

During evolution, survival against viruses would be favored by an immunological system which responded adequately to these pathogens. While this may be optimized against such invaders, the immunological system may mediate a hypernormal response to environmental antigens that cross-react with self antigens, giving rise to an autoimmune disease. In this respect, it has been possible to associate certain mouse and human histocompatibility antigens with autoimmune disease. The alleles DR 2, 3, and 4 are primarily associated with the conditions of hyperthyroidism and myasthenia gravis, where the antibodies are directed against thyroid-stimulating hormone and acetylcholine receptors, respectively. Whereas there is no direct evidence that viruses may play a role in the selection of variant immunologically competent populations, natural history may provide some indirect proof. Large variability in susceptibility or resistance to infectious diseases exists among genetically heterogeneous individuals in natural animal populations. The grouping based on these differences can often be equated to the same animal species located in separate geographical regions (Biozzi *et al.*, 1984). Although nonimmunological factors may also be involved, it has been clearly demonstrated that the principal functions of the immune system are subject to polygenic quantitative regulation since they can be drastically modified by bidirectional selective breeding (Biozzi *et al.*, 1980). It may, therefore, be possible that viruses exert selective pressure on the genetics of the host's immunological system.

## V. INTERFERON

One of the host's earliest responses to virus infection is to synthesize interferon. Studies with purified interferons have shown that both virus replication and cell multiplication may be inhibited (Knight, 1976; Gresser *et al.*, 1979). The early hopes for interferon as a treatment in disease must now be reassessed with care. The antiviral activity is no longer considered the most important biological activity. Moreover, a careful evaluation of the broader role of interferons in the regulation of the immune responses, cell growth, and differentiation is needed (Stewart, 1979).

The sensitivity to interferon varies with the type of cells. It may inhibit both normal and tumor cells (Gresser and Bourali, 1970), allogeneic lymphocytes and syngeneic bone marrow cells when transplanted into irradiated mice (Cerottini *et al.*, 1973), and regenerating liver cells in partially hepatectomized mice (Frayssinet *et al.*, 1973). At the cellular level, interferon can significantly alter the membrane, including an increased expression of certain cell surface antigens or receptors (Knight and Korant, 1977). Cultivation of several human B and T lymphoblastoid cell lines in the presence of  $\alpha$ -interferon resulted in the appearance of "lupus inclusions" (Rich, 1981). Similar inclusions are found in the glomerular endothelium and the peripheral blood lymphocytes of patients with systemic lupus erythematosus (SLE).

## A. Immune System

In animals, interferon has been assigned a number of toxic effects including leukopenia, thrombocytopenia, and hair loss. The level at which interferon intervenes may be multifold. Interferon, administered to an animal prior to antigenic challenge, suppresses both the primary antibody response (Chester *et al.*, 1973; Merigan *et al.*, 1975) and the induction of an anamnestic response (Brodeur and Merigan, 1975). It affects both the T-dependent and T-independent classes. Gisler *et al.* (1974) have also shown a direct suppression on B cells.

The studies of a number of groups (Virelizier *et al.*, 1977; Sonnenfeld *et al.*, 1977; Lucero *et al.*, 1980) have shown that  $\alpha$ -interferon suppresses the *in vitro* and *in vivo* immune response to sheep red blood cells.  $\gamma$ -Interferon was significantly (20–250 times) more suppressive than preparations of  $\alpha$ - or  $\beta$ -interferons. This is consistent with the hypothesis that  $\gamma$ -interferon acts as an immunoregulating lymphokine, suppressing antibody response. This would exert an antiproliferative effect

on these B cells, which would normally be proliferative in the response to the antigen.

In upper respiratory tract infections, interferon has a potential pathological role in the development of asthma (Hooks *et al.*, 1980; Sonnenfeld, 1980). There is a temporal relationship between the augmentation of histamine release and the induction of interferon in cultures (Hooks *et al.*, 1980). Additionally, interferons may serve to induce other cofactors associated with the reagenic response, such as IgE binding factors (Yodoi *et al.*, 1981).

### *B. Pathological Effects*

The problems addressed so far are due to the direct action of interferon on the different cell systems resulting in an immediate cellular change. However, it is possible that interferon can produce a lesion whose pathology is only revealed at a later stage. The most relevant studies from this point of view come from Gresser's group. They have shown that inoculation of newborn Swiss mice with less than 88 ng/day of interferon for 7 days was lethal (Gresser *et al.*, 1981). The livers from these animals were filled with fat vesicles and had decreased glycogen. There were no inflammatory infiltrates (Gresser *et al.*, 1975, 1981), but an inhibition of the maturation of different organs was apparent. Inoculation of a tenth of the lethal dose did not retard growth; however, glomerular lesions appeared from the twentieth day, even though the interferon treatment had stopped at the seventh day. The glomerular lesions were progressive and were followed (several days to weeks) by the deposition of IgG and C3 along the glomerular basement, similar to that observed in "immune complex" nephritis (Gresser *et al.*, 1976). Subsequent work with highly purified material has shown that interferon can produce glomerular lesions as early as the fourth day (Gresser *et al.*, 1981). Glomerulonephritis was also observed in rats treated with rat interferon (Gresser *et al.*, 1979).

Thus, interferon can be shown to be toxic when inoculated into animals under the appropriate conditions. Can viruses mimic this situation and produce a similar pathology, i.e., one that is not due to the direct action of the virus, but to virus-induced interferon? It is now well established that certain of the arenaviruses, such as lymphocytic choriomeningitis (LCM) virus, give rise to persistent infections in a variety of hosts, and high levels of interferon may be associated with these infections. Newborn mice infected with LCM virus rapidly develop a persistent infection. The surviving mice develop glomerulonephritis. If anti-interferon antibody is administered at the time of



infection, despite 100-fold increase in virus production, there is no kidney damage or thickening of the glomerular basement membrane (Ronco *et al.*, 1980).

The correlation between interferon production and pathogenicity could be taken even further. Infection of different inbred lines of mice with LCM virus gives different rates of mortality (Rivière *et al.*, 1980). Despite similar levels of virus multiplication, the quantity and duration of the interferonemia varied. There was a direct correlation between the amount of interferon induced by LCM virus and the extent of the disease. Moreover, strains of mice who failed to develop glomerulonephritis with LCM virus alone did so when interferon was injected (Woodrow *et al.*, 1982). Thus, there is a genetic control of the production of interferon in response to LCM virus.

The above studies indicate that the overproduction of interferon in certain virus infections is a basis for retarded virus pathology. Viruses are still the best inducers of interferon, and the production of interferon appears to be an integral part of the host response to virus infection. So, can levels of interferon be used as a diagnosis for chronic infections? Serum interferon levels may be elevated in certain chronic diseases, such as that in NZB/W and MRL mice, which develop an autoimmune disease like SLE (Skurkovich *et al.*, 1981). Inoculation of type 1 or 2 ( $\gamma$ ) interferon into these mice increased the incidence of anti-erythrocyte, anti-SS DNA, and anti-soluble nucleoprotein antibody, and increased the severity of renal lesions (Sergiescu *et al.*, 1979; Adam *et al.*, 1980; Engelman *et al.*, 1981). In man, increased interferon titers have also been described in autoimmune disease (Skurkovich and Eremkina, 1975; Hooks *et al.*, 1979). Further, the state (active/inactive) of the disease could be correlated with the presence of interferon (Preble *et al.*, 1982).

The studies considered so far reveal that the induction and magnitude of interferon synthesis depend upon a number of factors, including both the virus strains used and the genetics of the host. Although the toxicity levels for interferon have been established and the resulting lesions characterized histologically, little is known at the biochemical level. Inoculation of suckling mice with interferon leads to pronounced increases of lipids in hepatocytes (Gresser *et al.*, 1975, 1981). This is paralleled by the presence of abnormal tubular aggregates arising from the endoplasmic reticulum of the hepatocytes. This would suggest a modification in lipid biosynthesis. Zwingelstein *et al.* (1985) inoculated a mixture of  $\alpha$ - and  $\beta$ -interferons into newborn mice and found a decrease in phospholipid synthesis and an increase in triglyceride synthesis. This would imply that interferon treatment re-

sults in the inhibition of a component in phospholipid biosynthesis during the maturation of hepatocytes in suckling mice.

The major unanswered question in the various studies is whether the initial dysfunction induced by interferon is in the liver and kidney, leading the deposition of complexes, or whether the interferon acts directly on the immune system stimulating immune complex formation. Alternatively, the production of interferon may be linked to an autoimmune process, such as might be triggered by autoantibodies reacting with cell surface antigen. The importance of the observations on interferon toxicity is that viruses can induce levels of interferon which invoke a delayed pathology, even though the virus may have been eliminated.

## VI. DISRUPTION OF DIFFERENTIATED CELL FUNCTION

The replication strategy of certain viruses may have evolved around their interaction with the immunological system, enabling them to remain in cells, either as "silent" (latent) or persistent infections. *In vitro* studies indicate that cells from such infections have normal morphological appearance, growth, and cloning rates. The replication of the virus, or the mere presence of the virus in the cell, may interfere with differentiated cell functions. The level at which viruses may intervene in such functions may be considered on at least one of two levels:

1. Cell membrane receptors form the first part of a chain of reactions by which signals pass across the cell membrane into the cells (Hirata and Axelrod, 1980). Viruses can intervene in these systems either by reducing the number of receptor molecules expressed on the membrane, or by interfering with the passage of the signal across the membrane.

2. Differentiated cells may have ancillary or "luxury functions" (Oldstone *et al.*, 1982). During virus persistence, only the cells' vital functions are maintained and the ancillary functions, such as the synthesis of hormones, become inhibited. This may in turn lead to a metabolic disease.

In both of the above systems, due to the less stringent environment of the persistent infection, mutations in the virus genome accumulate (Holland *et al.*, 1982). In parallel, the cells may become either permanently or temporarily modified during the course of infection. Thus,

there may be a coevolution of both virus and cells (Ahmed *et al.*, 1981). In such circumstances, the continuing presence of the virus, once it has stamped its "imprint" on the cell, may no longer be necessary. We have tried to answer the following questions. (1) How do viruses interfere with differentiated cell function? (2) Is a certain level of virus expression within the cell necessary? (3) Do cells "cured" of virus infection retain a virus imprint?

#### A. Cell Receptor Function

The simplest way a virus can down-regulate a cell receptor function is to reduce the number of receptors at the cell surface. A number of viruses have chosen this method to disrupt differentiated cell function. Such phenomena have been described for both *in vitro* and *in vivo* systems. A few of the examples are cited here. Neuroblastoma cells persistently infected with measles virus have reduced amounts of the neurotransmitter-degrading acetylcholinesterase. Infection of DBA/2 mice with encephalomyocarditis virus (M variant) leads to both a decrease in the number of insulin receptors and a reduction in receptor affinity on liver membranes during the first few days after infection. This returns to normal levels in late infection (Kwanishi *et al.*, 1982).

In C6 rat glioma cells persistently infected by canine distemper virus there is a 50% reduction in the number of  $\beta$ -adrenergic receptors in the cell membrane (Koschel and Münzel, 1980). In contrast, persistent infection in the same cell line with either measles or rabies virus does not affect the number of  $\beta$ -adrenergic receptors, but does create an impairment of the specialized membrane-bound cellular functions (Koschel and Halbach, 1979; Halbach and Koschel, 1979; Münzel and Koschel, 1981). In this case, there is a large reduction in the accumulation of intracellular cAMP following isoproterenol stimulation of  $\beta$ -adrenergic receptors (Koschel and Halbach, 1979). Both measles and rabies viruses belong to the group of "enveloped" viruses, which integrate their glycoproteins into the cell plasma membrane. Can these foreign glycoproteins play a primary role in the dysfunction of the specialized membrane cellular function, by their mere physical presence in the membrane? Barret and Koschel (1983) have shown that addition of specific virus antiserum to C6 cells persistently infected with measles virus leads to a gradual loss of virus membrane antigen. This was accompanied by the recovery of the catecholamine-dependent  $\beta$ -adrenergic receptor-stimulated cAMP synthesis. They concluded that the impairment of this cell function was due to the insertion of viral antigens in the cell membrane, rather than their accumulation in the cytoplasm. These experiments would indicate that

the virus-infected glial cells can recover their membrane functions following antibody modulation.

Viruses have been shown to interact with the different cells of the immune system. Their ability to modify the host's immune response was first observed at the turn of the Century (Von Pirquet, 1908). Lymphocytes from individuals with acute measles virus infection respond poorly to a variety of mitogenic or antigenic stimuli *in vitro* (Zweiman *et al.*, 1981; Finkel and Dent, 1973; Whittle *et al.*, 1978; Hirsch *et al.*, 1981). They are also deficient in making chemotactic factors (Anderson *et al.*, 1976; Hirsch *et al.*, 1981).

Infection of lymphocytes by measles virus *in vitro* leads to a "silent" virus infection, in which measles virus gene products cannot be detected in the cells (Lucas *et al.*, 1978). Despite the lack of observable virus replication, a number of important functions of lymphocytes including NK cell killing and Ig synthesis are inhibited. The reduction of NK cell activity by measles virus infection of effector lymphocytes occurs within 24 hours of infection (Casali *et al.*, 1984), but does not affect antibody-dependent cell-mediated cytotoxicity (Galama *et al.*, 1980). Influenza virus has also been shown to give rise to an immunosuppressed state in humans (Lachmann and Peters, 1982). Resting human lymphocytes are permissive for influenza virus, but they maintain their structural integrity and viability. In contrast to measles, influenza virus-infected lymphocytes have normal NK cell activity but fail to synthesize immunoglobulin (Casali *et al.*, 1984).

There are several levels at which a virus can intervene to produce an immunosuppressed state, e.g., modification of cell-cell recognition, or proliferation. Comparison of measles and influenza virus-infected lymphocytes suggest that the block in cell function does not correlate with the level of virus expression in lymphocytes. This is in contrast to the observations with measles virus-infected C6 cells, where the dysfunction only occurred when virus antigen was present in the cell membrane (Barret and Koschel, 1983). The conclusion which can be drawn from these experiments is that there is no general mechanism by which viruses interfere with cell function. In each case, the virus has adapted a particular strategy for a given cell. It is unclear in the case of silently infected cells whether a low level of virus replication is required, or if certain gene products suffice.

### *B. Inhibition of "Luxury Functions"*

Differentiated cells may excrete products, such as hormones, which function on cells other than those which produce them. Noncytotoxic infections in these cells can lead to a decrease in the excretion of these

products without affecting the vital functions of the cells. The net results of such an inhibition may lead to a metabolic disease in the case of a hormone-producing cell, or to immunodysfunction if it is a cell of the immunological system.

The potential to initiate this type of infection is determined by a number of factors including the tropism of the virus, and ability of the virus to produce a noncytotoxic infection, or the ability of the host to modulate the infection. Oldstone and colleagues have shown that LCM virus can produce persistent infections in the anterior lobe of the murine pituitary gland and the beta cells of the pancreas (Oldstone *et al.*, 1982). In the pituitary gland, the virus replicates in growth hormone-producing cells. The resulting diminished synthesis of this hormone leads to retarded growth and hypoglycemia. In the persistently infected beta cells, virus antigen persists for months. Serum insulin levels are decreased, blood glucose levels are elevated, and glucose tolerance tests are abnormal. Similar results were obtained in hamsters infected with Venezuelan encephalitis virus (Rayfield *et al.*, 1981).

The cause of the virus-induced changes is not clear, but Notkins and Yoon (1984) have suggested that, for the pancreatic infections, there is an abnormality in the generation of cAMP. Studies of mice infected with Venezuelan equine encephalomyelitis virus show there is a decrease in the turnover of catecholamines in a number of regions of the brain. Certain regions returned to normal 12–18 days after infection, but recovery was slower in other regions (Lima *et al.*, 1983).

In these studies, no information is available on the level of virus expression and the inhibition of the luxury function. Lymphocytes infected either “silently” with measles virus or productively with influenza fail both to proliferate and to synthesize immunoglobulins (Casali *et al.*, 1984). However, when the lymphocytes were infected after immunoglobulin synthesis had started, no inhibition was observed. Measles virus acts on lymphocytes at an early stage but cannot block fully differentiated cells from synthesizing immunoglobulin.

### *C. Immunomodulation of Persistently Infected Cells*

During virus infection, the host's immunological response limits further dissemination of the virus. At the cellular level, virus antigens can be modulated or “stripped” from the cell membrane by antibodies (Joseph and Oldstone, 1978). Rustigan (1966) found that antibody treatment of measles virus persistent infections of HeLa cells reduced the expression of virus antigens at the cell membrane, but did not lead to the curing of the virus infection. In contrast, cultivation of C6 glial

cells persistently infected with measles virus in the presence of virus antibody leads to the complete disappearance of virus antigens (Barrett *et al.*, 1985). Removal of the antibody leads to the reexpression of the virus antigens. When the cells were cloned after antibody treatment, although the cell clones were initially negative for measles virus expression, one third became positive when cultivated for periods up to 9 months. Of the remainder, half could be induced to express virus antigens, whereas the remainder could not. In similar experiments with cells of non-nervous tissue origin, HeLa and Vero, despite modulation of the expression of virus surface antigens, Barrett *et al.* were unable to suppress the synthesis of internal virus antigens completely. Thus, the modulation by specific antibody of virus-infected nervous or nonnervous tissue could have different outcomes. This may be of some consequence if a defined level of virus antigen is required to interfere with differentiated cell function.

#### D. "Cured" Cells

Cell clones which are negative for virus expression have been obtained from a variety of persistently infected cultures. Barrett *et al.* (1985) suggested that such cells obtained from measles virus-infected C6 glioma cells are not really cured, but contain latent virus which may be activated by long-term cell passage. Alternatively, this may be a property of cells of nervous tissue origin. In either case, the fundamental problem is whether, in the absence of virus or virus expression, the cells retain an "imprint" of the original virus infection; i.e., does the virus irreversibly change its host?

Studies from a number of laboratories would suggest that viruses can permanently modify certain cellular characteristics. L cells persistently infected with reovirus, when treated with virus antibody, gave rise to cell clones in which virus could not be detected (Ahmed *et al.*, 1981). The cells differed from the parental cell line in their sensitivity to superinfection. They gave rise to persistent infections with either the wild type or virus originating from the persistently infected culture. In contrast, the same viruses gave lytic infections in the parental L cells. The cured cell lines were morphologically distinct from the parental line, being larger and less fibroblastic-like. They had an altered cytoskeleton and contained a large number of lysosome-like structures in the cytoplasm. Unlike the parent line, the cured cells were also resistant to infection with vesicular stomatitis virus. There are further examples in which uninfected cells derived from persistent infections have altered susceptibility to virus infection; e.g., HeLa cells derived from carrier cultures of poliovirus or Coxsackie A9 virus

infections are more resistant to superinfection than the parental line (Ackerman and Katz, 1955; Vogt and Dulbecco, 1958; Takemoto and Habel, 1959).

In the experiments cited above, it is not clear if the conditions favor the selection of uninfected variant cells. To resolve this question, Ishida *et al.* (1964) made use of the observation that HeLa cells persistently infected with Sendai virus contained specific chromosomal modifications. This was correlated with a reduction in the levels of alkaline phosphatase (Ebina *et al.*, 1969). In the initial infection, the majority of the cells gave rise to a persistent infection and had a modified karyotype within the first 40 hours of infection. In this system, it is evident that the observed phenomenon is not due to a selection of variants, as the changes occurred in virtually all the cells.

Specific karyotype modifications have been observed in BGM cells persistently infected with measles virus (Patet and Wild, unpublished observations). Compared to the uninfected cells, the plasma membrane is modified during the persistent infection. The fatty acid composition of the cell membrane of the infected cultures has an increased palmitic and arachidonic acid content (Anderton *et al.*, 1981), and the cells have a perturbation in fatty acid metabolism. Compared to uninfected cells, fatty acids accumulate in the triglyceride fraction due to a slower turnover of the latter (Anderton *et al.*, 1983). The changes in the plasma membrane in the persistently infected cells favor the exposure of certain cellular proteins previously not accessible to the exterior (Wild *et al.*, 1981). Approximately half of the cell clones obtained in the absence of virus antibody did not contain virus or virus antigen. However, they still retained the modifications associated with the original persistent infection, i.e., modified fatty acid metabolism, and membrane and karyotype changes. Unfortunately, in this system it is not possible to evaluate whether these modifications are sufficient to affect the luxury functions of differentiated cells.

*In vivo* evaluation of animal models or human disease for a retarded pathology after the elimination of the virus is more difficult. In the LCM virus/mouse models for the inhibition of hormones, Oldstone *et al.* (1982) described the presence of virus antigens in the hormone-secreting tissue. In contrast, infection of mice with canine distemper virus leads to a neurological infection in which a proportion of the surviving mice become obese (Bernard *et al.*, 1983; Lyons *et al.*, 1982). This state is preceded by a hyperinsulinemic phase (Bernard and Wild, unpublished observation). Body weights of obese animals, 16–20 weeks after infection, were comparable to those reported for genetically obese mice and for mice rendered obese by hypothalamic lesions. Brain tissue from the obese mice showed no overt pathology and virus

could not be detected in this or other tissues. The levels of dopamine and norepinephrine were two to three times lower in obese mouse brain than in controls (Lyons *et al.*, 1982). Thus, a primary pathological process involving virus-induced destruction or dysfunction of select groups of neurons, resulting in defective catecholamine synthesis or release, may be critical in the development of the syndrome. The role of the virus in the obesity syndrome still remains to be ascertained.

The evolution of the immune system dictates that it develops a response to eliminate foreign matter. At the same time, virus survival depends on their ability to outmaneuver this response. In the present section, we have seen how virus-specific antibody can limit cellular virus expression and may even eliminate the virus completely. This may depend on the origin of the cell or the nature of the infection. In the noncytotoxic infections there will be varying degrees of virus expression, and the quantitative aspects may, in turn, influence differentiated cell functions. In contrast, other cells such as lymphocytes producing immunoglobulins, once the differentiated function has started, cannot be deprogrammed even when large amounts of virus antigens are synthesized.

## VII. CONCLUSIONS AND PERSPECTIVES

In our introduction, we presented the concept that virus may no longer be present when the pathology appears. We have examined the effect of viruses in inducing modifications in chromosomes, immune system interactions, cell metabolism, etc., to support such a hypothesis. A literature survey revealed a volume of papers to support this conclusion. A number of studies have described subtle alterations of biological functions of infected cells or organisms, but this has not been followed up as a subject in its own right.

For obvious reasons, virologists initially studied the agents causing acute diseases. However, when vaccines were available to protect the population against the major epidemics, chronic, recurrent, or persistent infections came into focus. Viruses are everywhere and all organisms are permanently exposed to them. The result of this contact depends on the particular moment at which a given cell is exposed to the virus. Rubella, for example, provokes a very benign disease in adults, but can induce abnormalities in the differentiating fetus. Although interferon is active against virus aggression, excess interferon has a toxic effect and can block differentiation.

After recovery from a virus infection does the virus "incident" be-



come forgotten? There is always the immunological memory, but are there other "memories"? The capacity of several viruses to act as cellular mutagens by modifying chromosomes is well established, whatever the molecular mechanism may be. Mutations at the cellular level were carefully analyzed for their tumorigenic potential, but much less attention was devoted to other metabolic errors, even though modified enzyme activities have been observed.

Even if the mechanism of cell killing in acute virus infections is little understood, it is known that cellular functions are greatly modified. However, when the cell is not killed, are all the metabolic changes reversible? From the papers quoted in this review, it is evident that both situations can arise depending upon the system analyzed. Epigenetic modifications may behave as if they have become genomic.

A new hypothesis is only useful if it offers an experimental approach. In human pathology, if viruses act only as a trigger, it is difficult to trace the origin of the disease to the original event. This is even more true if several agents trigger a similar response. A systematic analysis for immunological footprints may throw some light on the problem. Epidemiological studies on the frequency and patterns of "nonvirus" diseases following vaccination programs may elucidate the role of certain viruses. Such an attempt was made by Ronne (1985). He found an increase in several diseases (immunoreactive, degenerative diseases of bone and cartilage, and certain tumors) in the section of the Danish adult population which had a history of inapparent measles infection in childhood. These obviously represent one end of the spectrum. Presumably, further advances may be made by studying intermediate situations, in which persistent virus infections are established. Careful study of various dysfunctions in the latter may reveal the indirect role that viruses may play in disease. Obviously, more effort is needed in the construction of both *in vitro* and *in vivo* models. Such systems should be capable of answering the problems of how, after a temporary residence in a cell, organ, or organism, a virus can leave its imprint on the system. Thus, the correlation between such virus markers and the late onset of disease awaits clarification.

#### REFERENCES

- Ackerman, W. W., and Kurtz, H. (1955). *J. Exp. Med.* **102**, 555.  
Adam, C., Thoua, Y., Ronco, P., Verroust, P., Tovey, M., and Morel-Maroger, L. (1980). *Clin. Exp. Immunol.* **40**, 373.  
Ahmed, R., and Oldstone, M. (1984). In "Concepts in Viral Pathogenesis" (A. L. Notkins and M. B. A. Oldstone, eds.), p. 231. Springer-Verlag, Berlin and New York.

- Ahmed, R., Canning, W. M., Kauffman, R. S., Sharpe, A. H., Hallum, J. V., and Fields, B. N. (1981). *Cell* **25**, 325.
- Allison, A. C., and Paton, G. R. (1965). *Nature (London)* **207**, 1170.
- Altman, A., and Katz, D. H. (1982). *Adv. Immunol.* **33**, 73.
- Anderson, R., Rabson, A. R., Sher, R., Koornhof, H. J., and Bact, D. (1976). *J. Pediatr.* **89**, 27.
- Anderton, P., Wild, T. F., and Zwingelstein, G. (1981). *Biochem. Biophys. Res. Commun.* **103**, 285.
- Anderton, P., Wild, T. F., and Zwingelstein, G. (1983). *Biochem. J.* **214**, 665.
- Aula, P., and Nichols, W. W. (1968). *Exp. Cell Res.* **51**, 595.
- Barrett, P. N., and Koschel, K. (1983). *Virology* **127**, 299.
- Barrett, P. N., Koschel, K., Carter, M., and ter Meulen, V. (1985). *J. Gen. Virol.* **66**, 1411.
- Bellett, A. J., Waldron-Stevens, L. K., Braithwaite, A. W., and Cheetham, B. F. (1982). *Chromosoma* **84**, 571.
- Bernard, A., Wild, T. F., and Tripier, M. F. (1983). *J. Gen. Virol.* **64**, 1571.
- Biozzi, G., Siqueira, M., Stiffel, C., Ibanez, O. M., Mouton, D., and Ferreira, V. C. A. (1980). In "Immunology 80—Progress in Immunology IV" (M. Fougereau and J. Dausset, eds.), p. 432. Academic Press, New York.
- Biozzi, G., Mouton, D., Stiffel, C., and Bouthillier, Y. (1984). *Adv. Immunol.* **36**, 189.
- Boué, J. G., Boué, A., Moorhead, P. S., and Plotkin, S. A. (1964). *C.R. Acad. Sci. Paris* **259**, 687.
- Brodeur, B. R., and Merigan, T. C. (1975). *J. Immunol.* **114**, 1323.
- Butchko, G. M., Armstrong, R. B., Martin, W. J., and Ennis, F. A. (1978). *Nature (London)* **271**, 66.
- Calafat, J., Janssen, H., Demant, P., Hilgers, J., and Zavada, J. (1983). *J. Gen. Virol.* **64**, 1241.
- Cantell, K., Saksela, E., and Aula, P. (1966). *Ann. Med. Exp. Fenn.* **44**, 255.
- Casali, P., Rice, G. P. A., and Oldstone, M. B. A. (1984). *J. Exp. Med.* **159**, 1322.
- Cerottini, J. C., Brunner, K. T., Lindahl, P., and Gresser, I. (1973). *Nature (London) New Biol.* **242**, 152.
- Chester, T. J., Pauker, K., and Merigan, T. C. (1973). *Nature (London)* **246**, 92.
- Crawford, L., Leppard, K., Lane, D., and Harlow, E. (1982). *J. Virol.* **42**, 612.
- Csonka, E., Ruzicska, P., and Koch, A. S. (1975). *Acta Microbiol. Acad. Sci. Hung.* **22**, 41.
- Damonte, E. B., De Salum, S. B., Larrida, I., and Coto, G. E. (1979). *Medicina (Buenos Aires)* **39**, 589.
- Day, R. N. (1966). *Am. J. Hum. Genet.* **18**, 70.
- Dighiero, G., Guilbert, B., and Avrameas, S. (1982). *J. Immunol.* **128**, 2788.
- Drzeniek, R., and Rott, R. (1969). *Int. Arch. Allergy* **36**, 146.
- Duc Dodon, M., and Quash, G. A. (1981). *Immunology* **42**, 401.
- Duc Dodon, M., Gazzolo, L., Quash, G. A., and Wild, T. F. (1982). *J. Gen. Virol.* **63**, 441.
- Dutton, R. W. (1975). *Transplant. Rev.* **23**, 66.
- Eaton, M. D. (1980). *Infect. Immun.* **27**, 855.
- Ebina, T., Takahashi, K., Homma, M., and Ishida, N. (1969). *Virology* **39**, 597.
- Engelman, E. G., Sonnenfeld, G., Dauphinee, M., Greenspan, J. S., Talal, N., McDevitt, H. O., and Merigan, T. C. (1981). *Arthritis Rheum.* **24**, 1396.
- Epplen, J. T., Vogel, W., and Schwöbel, W. (1977). *Zentralbl. Bakteriolog. Hyg. I. Abt. Orig.* **239**, 149.
- Finkel, A., and Dent, P. B. (1973). *Cell. Immunol.* **6**, 41.
- Fong, S., Tsaukas, C. D., Frincke, L. A., Lawrence, S. K., Holbrook, T. L., Vaughan, J. H., and Carson, D. A. (1981). *J. Immunol.* **126**, 910.
- Frayssinet, C., Gresser, I., Tovey, M., and Lindahl, P. (1973). *Nature (London)* **245**, 146.

- Fujinami, R. S., Oldstone, M. B. A., Wroblewska, Z., Frankel, M. E., and Koprowski, H. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2346.
- Galama, J., Ubels-Postma, J. C., and Lucas, C. J. (1980). *Cell. Immunol.* **50**, 405.
- Galloway, D. A., and McDougall, J. K. (1983). *Nature (London)* **302**, 21-24.
- Galloway, G., Leung, A. Y.-T., Hunneyball, I. M., and Stanworth, D. R. (1983). *Immunology* **49**, 511.
- Gibson, M., Tiensiwakul, P., and Khoobyarian, N. (1982). *Cell. Immunol.* **73**, 397.
- Gisler, R. H., Lindahl, P., and Gresser, I. (1974). *J. Immunol.* **113**, 438.
- Golubovky, M. D., and Plus, N. (1982). *Mutat. Res.* **103**, 29.
- Goodman-Snitkov, G. W., and McSharry, J. J. (1980a). *J. Virol.* **35**, 757.
- Goodman-Snitkov, G. W., and McSharry, J. J. (1980b). *Infect. Immun.* **38**, 1242.
- Gorshunova, L. P., and Mikhaïlova, G. R. (1976). *Vopr. Virusol.* **11**, 521.
- Gould, E. A., Chanas, A. C., Buckley, A., and Clegg, C. S. (1983). *Infect. Immun.* **41**, 774.
- Gresser, I., and Bourali, C. (1970). *J. Natl. Cancer Inst.* **45**, 365.
- Gresser, I., Tovey, M. G., Maury, C., and Chouroulinkov, I. (1975). *Nature (London)* **258**, 76.
- Gresser, I., Morel-Maroger, L., Maury, C., Tovey, M. G., and Pontillon, F. (1976). *Nature (London)* **263**, 420.
- Gresser, I., DeMaeyer-Guignard, J., Tovey, M. G., and Demaeyer, E. (1979). *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5308.
- Gresser, I., Aguet, M., Morel-Maroger, L., Woodrow, D., and Puvion-Dutilleul, F. (1981). *Am. J. Pathol.* **102**, 396.
- Haase, A. T., Gantz, D., Eble, B., Walker, D., Stowring, L., Ventura, P., Blum, H., Wietgreffe, S., Zapancic, M., Tourtellotte, W., Gibbs, C. J., Norrby, E., and Rozenblatt, S. (1985). *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3020.
- Haire, M. (1972). *Clin. Exp. Immunol.* **12**, 335.
- Halbach, M., and Koschel, K. (1979). *J. Gen. Virol.* **42**, 615.
- Hampar, B., and Ellison, S. A. (1961). *Nature (London)* **192**, 145.
- Hand, R. (1976). *Virology* **71**, 609.
- Haspel, M. V., Onodera, T., Prabhakar, B. S., Horita, M., Suzuki, H., and Notkins, A. L. (1983a). *Science* **220**, 304.
- Haspel, M. V., Onodera, T., Prabhakar, B. S., McClintock, P. R., Essani, K., Ray, U. R., Yagihashi, S., and Notkins, A. L. (1983b). *Nature (London)* **303**, 73.
- Hirata, F., and Axelrod, J. (1980). *Science* **209**, 1082.
- Hirsch, R. L., Mokhtarian, F., Griffin, D. E., Brooks, B. R., Hess, J., and Johnson, R. T. (1981). *Clin. Immunol. Immunopathol.* **21**, 341.
- Hirsch, R. S., and Proffitt, M. R. (1975). In "Viral Immunology and Immunopathology" (A. L. Notkins, ed.), p. 419. Academic Press, New York.
- Hoffman, R. M., Jacobsen, I. J., and Erbe, R. W. (1978). *Biochem. Biophys. Res. Commun.* **82**, 228.
- Holland, J., Spindler, K., Horodyski, F., Grabau, E., Nichol, S., and Vandefol, S. (1982). *Science* **215**, 1577.
- Homma, M., Ohira, M., and Ishida, N. (1968). *Virology* **34**, 60.
- Hooks, J. J., Moutsopoulos, H. M., Geis, S. A., Stahl, N. I., and Decker, J. L. (1979). *N. Engl. J. Med.* **301**, 5.
- Hooks, J. J., Moutsopoulos, H. M., and Notkins, A. L. (1980). *Ann. N.Y. Acad. Sci.* **350**, 21.
- Howard, M., and Paul, W. E. (1983). *Annu. Rev. Immunol.* **1**, 307.
- Huang, C. C. (1971). *Mamm. Chromosomes Newslett.* **12**, 17.
- Huppert, J., and Wild, T. F. (1984). *Ann. Virol.* **135**, 327.
- Ilinskikh, N. N., and Ilinskich, I. N. (1976). *Tsitol. Genet.* **10**, 331.

- Ishida, N., Homma, M., Osato, T., Hinuma, Y., and Miyamoto, T. (1964). *Virology* **24**, 320.
- Joseph, B. S., and Oldstone, M. B. A. (1975). *J. Exp. Med.* **142**, 864.
- Karsenti, E., Guilbert, B., Bornens, M., and Avrameas, S. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3997.
- Knight, E. (1976). *Nature (London)* **262**, 302.
- Knight, Jr., E., and Korant, B. D. (1977). *Biochem. Biophys. Res. Commun.* **74**, 707.
- Koprowski, H., Ponten, J. A., Jensen, F., Ravdin, R. G., Moorhead, P., and Saksela, E. (1962). *J. Cell. Comp. Physiol.* **59**, 281.
- Koschel, K., and Halbach, M. (1979). *J. Gen. Virol.* **42**, 627.
- Koschel, K., and Münzel, P. (1980). *J. Gen. Virol.* **47**, 513.
- Kurki, P., Virtanen, I., Stenman, S., and Linder, E. (1978). *Clin. Immunol. Immunopathol.* **11**, 379.
- Kwanishi, K., Kubota, M., and Yagyu, F. (1982). *Endocrinology Jpn.* **29**, 407.
- Lachmann, P., and Peters, D. K. (1982). In "Clinical Aspects of Immunology," 4th Ed., Vol. 2. Blackwell, Oxford.
- Landa, Z., Svoboda, J., and Jirasek, J. (1962). *Folia Biol.* **8**, 12.
- Lidman, K., Biberfeld, G., Fagreu, A., Norberg, R., Torstensson, R., Utter, G., Carlsson, L., Luca, J., and Lindberg, U. (1976). *Clin. Exp. Immunol.* **24**, 266.
- Lima, L., D'iaz Borges, J. M., and Walder, R. (1983). *J. Neurosci. Res.* **10**, 61.
- Linder, E., Kurki, P., and Andersson, L. C. (1979). *Clin. Immunol. Immunopharmacol.* **14**, 411.
- Lipton, H. L., and Dal Canto, M. (1979). *J. Neurol. Sci.* **42**, 391.
- Lodish, H., and Porter, M. (1980). *Cell* **19**, 161.
- Lucas, C. J., Ubels-Postma, J., Galama, J. M., and Rezee, P. (1978). *Cell. Immunol.* **37**, 448.
- Lucero, M. A., Wietzerbin, J., Stefanos, S., Billardon, C., Falcoff, E., and Fridman, W. H. (1980). *Cell. Immunol.* **54**, 58.
- Luleci, G., Sakéizèi, M., and Gunalp, A. (1980). *Acta Virol. (Prague)* **24**, 341.
- Lyons, M. J., Faust, I. M., Hemmes, R. B., Buskirk, D. R., Hirsch, J., and Zabriskie, J. B. (1982). *Science* **216**, 82.
- Majer, M., Herrmann, A., and Mauler, R. (1977). *Arch. Virol.* **54**, 255.
- Marengo, C., Mbikay, M., Weber, J., and Thirion, J. P. (1981). *J. Virol.* **38**, 184.
- Marshak, M., Varshaver, I., and Shapiro, N. B. (1975). *Mutat. Res.* **30**, 383.
- Massanari, R. M., Paterson, P. Y., and Lipton, H. L. (1979). *J. Infect. Dis.* **139**, 297.
- Merigan, T. C., Chester, T. J., and Pauker, K. (1975). In "Effects of Interferon on Cells, Viruses and the Immune System" (A. Geraldès, ed.), p. 347. Academic Press, New York.
- Mims, C. A., ed. (1982). In "The Pathogenesis of Infectious Diseases." Academic Press, London.
- Munyon, W. M., Kraiselburd, E., Davis, D., and Mann, J. (1971). *J. Virol.* **7**, 813.
- Münzel, P., and Koschel, K. (1981). *Biochem. Biophys. Res. Commun.* **101**, 1241.
- Muraguchi, A., Butler, J. L., Kehre, J. H., and Fauci, A. S. (1983). *J. Exp. Med.* **157**, 530.
- Nichols, W. W. (1962). *Hereditas* **48**, 367.
- Nichols, W. W. (1970). *Annu. Rev. Microbiol.* **24**, 479.
- Nichols, W. W., and Heneen, W. K. (1964). *Hereditas* **52**, 402.
- Norrby, E., Levan, A., and Nichols, W. W. (1966). *Exp. Cell Res.* **41**, 483.
- Notkins, A. L., and Yoon, N. W. (1984). In "Concepts in Viral Pathogenesis" (A. L. Notkins and M. B. A. Oldstone, eds.), p. 241. Springer-Verlag, Berlin and New York.
- Oldstone, M. B. A., Sinha, Y. N., Blount, P., Tishon, A., Rodriguez, M., von Wedel, R., and Lampert, P. W. (1982). *Science* **218**, 1125.

- O'Neill, F. J., and Rapp, F. (1971). *Virology* **44**, 544.
- Panitch, H., Petursson, G., Georgsson, G., Pálsson, P., and Nathanson, N. (1976). *Lab. Invest.* **35**, 452.
- Pilon, L., Royal, A., and Langelier, Y. (1985). *J. Gen. Virol.* **66**, 259.
- Preble, O. T., Black, R. J., Friedman, R. M., Klippel, J. H., and Vilcek, J. (1982). *Science* **216**, 429.
- Pruslin, F. H., and Rodman, T. C. (1978). *Infect. Immun.* **19**, 1104.
- Rayfield, E. J., Seto, Y., Walsh, S., and McEvoy, R. C. (1981). *J. Clin. Invest.* **68**, 1172.
- Rich, S. A. (1981). *Science* **213**, 772.
- Rivière, Y., Gresser, I., Guillon, J.-C., Bandu, M.-T., and Ronco, P. (1980). *J. Exp. Med.* **152**, 633.
- Robinson, A., and Puck, T. T. (1967). *Am. J. Hum. Genet.* **19**, 112.
- Ronco, P., Woodrow, D., Rivière, Y., Moss, J., and Verroust, P. (1980). *Lab. Invest.* **43**, 37.
- Ronne, T. (1985). *Lancet Jan.* **5**, 1.
- Rustigan, R. (1966). *J. Bacteriol.* **92**, 1792.
- Sablina, O. V. (1978). *Genetika* **14**, 1919.
- Schlehofer, J. R., and zur Hausen, H. (1982). *Virology* **122**, 471.
- Schwöbel, W., Vogel, W., and Epplen, J. T. (1977). *Zentral Bakteriell. Hyg. I. Abt. Orig.* **239**, 141.
- Sergiescu, D., Cerutti, I., Efthymiou, E., Kahan, A., and Chany, C. (1979). *Biomedicine* **31**, 48.
- Shein, H. N., and Enders, J. F. (1962). *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1164.
- Shelton, I. H., Kasupski, G. J., Jr., Oblin, C., and Hand, R. (1981). *Can. J. Biochem.* **59**, 122.
- Sheshberadaran, H., and Norrby, E. (1984). *J. Virol.* **52**, 995.
- Skurkovich, S. V., and Eremkina, E. I. (1975). *Ann. Allergy* **35**, 356.
- Skurkovich, S. V., Steinberg, A., Skurkovich, B., and Harrell, S. (1981). *Annu. Int. Congr. Interferon Res.*, 2nd.
- Sonnenfeld, G. (1980). *Lymphokine Rep.* **1**, 113.
- Sonnenfeld, G., Mandel, A. D., and Merigan, T. C. (1977). *Cell. Immunol.* **34**, 193.
- Steck, A. J., Tschannen, R., and Schäfer, R. (1981). *Neuroimmunology* **1**, 117.
- Stewart, W. E. (1979). In "The Interferon System." Springer-Verlag, Berlin and New York.
- Stich, H. F., and Yohn, D. S. (1970). *Prog. Med. Virol.* **12**, 78.
- Stoller, A., and Collmann, R. D. (1965). *Lancet* **ii**, 1221.
- Takemoto, K. K., and Habel, K. (1959). *Virology* **7**, 28.
- Thadani, M. A., and Polasa, H. (1979a). *Hum. Genet.* **49**, 97.
- Thadani, M. A., and Polasa, H. (1979b). *Hum. Genet.* **51**, 253.
- Theile, M., Scherneck, S., and Geissler, E. (1976). *Mutat. Res.* **37**, 111.
- Theile, M., Strauss, M., Luebbe, L., and Scherneck, S., Krause, H., and Geissler, E. (1979). *Cold Spring Harbor Symp. Quant. Biol.* **44**, 377.
- Theofilopoulos, A. N., and Dixon, F. J. (1982). *Am. J. Pathol.* **108**, 321.
- Toh, B. H., Yildiz, A., Sotelo, J., Osung, O., Holborow, E. J., Kanakoudi, F., and Small, J. V. (1979). *Clin. Exp. Immunol.* **37**, 76.
- Tonetti, G., Oldstone, M. B. A., and Dixon, F. J. (1970). *J. Exp. Med.* **132**, 89.
- Tschannen, R., Steck, A. J., and Schäfer, R. (1979). *Neurosci. Lett.* **15**, 295.
- Varshaver, N. B., Marshak, M. I., and Luss, E. (1977). *Mutat. Res.* **43**, 263.
- Virelizier, J. L., Chan, E. L., and Allison, A. C. (1977). *Clin. Exp. Immunol.* **30**, 299.
- Vogt, M., and Dulbecco, R. (1958). *Virology* **5**, 425.
- von Pirquet, C. E. (1908). *Dtsch. Med. Wochenschr.* **34**, 1297.
- Wardley, R. C. (1982). *Immunology* **46**, 215.
- Watanabe, R., Wege, H., and ter Meulen, V. (1983). *Nature (London)* **305**, 150.

- Whittle, H. C., Dossetor, J., Oduloju, A., Bryceson, D. M., and Greenwood, B. M. (1978). *J. Clin. Invest.* **62**, 678.
- Wild, T. F., and Greenland, T. (1979). *Intervirology* **11**, 275.
- Wild, T. F., Bernard, A., and Greenland, T. (1981). *Arch. Virology* **67**, 297.
- Woodrow, D., Ronco, P., Rivière, Y., Moss, J., and Gresser, I. (1982). *Br. J. Pathol.* **138**, 325.
- Yamanishi, K., Matsunaga, Y., Ogino, T., and Lopetegui, P. (1981). *J. Gen. Virol.* **56**, 421.
- Yodoi, J., Hirashima, M., Bloom, B. R., and Ishizaka, K. (1981). *J. Immunol.* **127**, 1579.
- Zavada, J. (1982). *J. Gen. Virol.* **63**, 15.
- Zweiman, B., Pappagianis, D., Maibach, H., and Hildreth, E. A. (1971). *Int. Arch. Allergy Appl. Immunol.* **40**, 834.
- Zwengelstein, G., Meister, R., Malak, N. A., Maury, C., and Gresser, I. (1985). *J. Interferon Res.* **5**, 315.
- zur Hausen, H. (1980). *Adv. Cancer Res.* **33**, 77.