Persistent infections

Persistent infection of the mouse with the virus of lymphocytic choriomeningitis

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Less than 20 years ago the physical and chemical characterization of a virus required knowledge, skill, experience, and patience. Today the same task has become a matter of routine, and an ever increasing number of viruses is sufficiently characterized to allow hiding ignorance behind reasonable speculations. To these the virus of lymphocytic choriomeningitis does not belong, and although this agent and its interaction with the mouse has contributed much to our understanding of biological and medical phenomena, such as immunological tolerance, latent viral infections, immunopathology of virus diseases, and slow virus diseases (Lehmann-Grube, 1971), little is known of its physico-chemical constitution. In the past, theoretical considerations have often outrun well founded knowledge and, rather than guiding further experimentation, they have often led into blind alleys. In this paper, I wish to present a few facts about this virus and at the same time draw attention to some of the gaps in knowledge which must be bridged before further interpretations of biological phenomena may profitably be attempted.

In order to avoid misunderstandings, it is necessary to start with a few definitions. Lymphocytic choriomeningitis (LCM) is the term used for any illness caused by the LCM virus. Thus, LCM is not a clinical entity and further characterization is achieved by referring to such syndromes as LCM meningitis. It is of great importance to draw a clear distinction between infection and disease, the former meaning entry of an agent into a cell or an organism, followed by multiplication, whereas the latter refers to all abnormal changes caused directly or indirectly by the multiplying pathogen. It goes without saying that infection is the prerequisite for an infectious disease and that not every infection is followed by illness. Indeed, numerous agents which are highly infectious may remain inapparent and rarely or never cause clinical signs. Such inapparent infections

are either subclinical, that is to say, they run their usual courses with final elimination of the agent and pathological alterations remain below the threshold of detection or are latent. A latent infection is one which is both inapparent and persistent. This definition, introduced by Andrewes (1958), seems to me still the best one available and I shall adhere to it, although one obvious disadvantage has to be mentioned, namely, the difficulty, if not impossibility, to prove the absence of all pathological alterations in the individual case (Lehmann-Grube, 1967a). Persistent infection is synonymous with carrier state and it denotes the lifelong coexistence of an agent with its host, regardless of whether or not clinical signs develop. A neonatal carrier is one which has received the virus—either by artificial means or by contact with infected litter mates—soon after birth. In a congenital carrier the virus is transmitted from the persistently infected mother, presumably at the very beginning of its ontogenetic development.

Immunity is the altered reactivity of an organism due to contact with an antigen; it contrasts with immunological tolerance. One, but not the only, expression of immunity is specific protection towards an infectious pathogen.

The basic phenomena which are observed when *Mus musculus* makes contact with LCM virus are shown diagrammatically in Figure 1. Infection of the immature mouse, ie, infection before or soon after birth, is followed by a lifelong viral carrier state. In contrast, if the virus is introduced into the adult animal, illness ensues which, depending on the virus strain, dose, and route of inoculation, leads either to death or recovery; in the latter case the virus is eliminated and protection against reinfection develops (Traub, 1935, 1936a, b, and c, 1938, 1939; Haas, 1954; Rowe, 1954). The significance of the coexistence of the virus with the mouse was recognized by Burnet and Fenner (1949). Together with erythrocyte chimerism in cattle twins (Owen, 1945) the

Fig. 1 Basic phenomena following infection of the mouse with LCM virus.

persistent infection of the mouse with the LCM virus formed the basis of the concept of self-recognition which later was to develop into one of the most intriguing and fruitful themes of modern immunology: immunological tolerance (Billingham, Brent, and Medawar, 1953). It was also Burnet (1955) who stressed that the coexistence of virus and host could only be perpetuated if the virus had 'virtually no power to damage the embryonic tissues in which it multiplies'. The finishing touch to this picture was applied by Hotchin (1962) who interpreted disease and death developing in mice infected after maturation as an allergy towards virus-induced new antigens.

Today, I wish to concentrate on two questions: first, is it true that the LCM virus is harmless for the mouse cell which it has invaded; and secondly, is the hypothesis that virus-specific immunological tolerance is the basis of the mechanism which prevents multiplying foreign antigen from being eliminated by the host still compatible with current knowledge? In accordance with the theme of this talk, disease in mice as a consequence of acute infection of the adult animal is only to be touched upon where necessary for discussing the mechanism underlying persistency.

Let us first consider the events which follow the introduction of the LCM virus into a culture of strain 'L' mouse cells (Fig. 2). The latent period is long, it is

independent of the dose and it takes eight hours before newly produced infectious virus becomes demonstrable. Infectivity then rises steeply for

Fig. 2 Multiplication of LCM virus, strain WE, in L cell monolayer cultures. Closed and open symbols denote cell-associated and released infectious virus, respectively. Different shapes of symbols refer to different experiments (Lehmann-Grube and Slenczka, 1967). Titrations were performed in L cell tube cultures according to Lehmann-Grube and Hesse (1967).

Fig. 3 Electron micrograph of L cells 48 hours after infection with LCM virus, strain WE; scale represents 1 μ m (kindly supplied by Dr Kl. Mannweiler, Hamburg).

Together with infectious virus, antigens, which are demonstrable by complement fixation, immunodiffusion, or immunofluorescence, are produced. On an Ouchterlony plate, two bands (Bro-Jørgensen, 1971; Lehmann-Grube, unpublished), sometimes more (Barlow and Mustico, 1966; Simon, 1970; Chastel, 1970) can be distinguished. Between 24 and 36 hours after infection, virions are seen budding from cell membranes by electron microscopy (Fig. 3). They are pleomorphic, ranging in size from 40 to over 300 nm, and contain electron-dense granules (Dalton, Rowe, Smith, Wilsnack, and Pugh, 1968; Mannweiler and Lehmann-Grube, to be published). Because of this appearance LCM virus—together with other similar agents—has been given the

Little is known of the intracellular events which culminate in the maturation of new virions. Not much more can be said of the relationship between the virion as seen with the electron microscope and assayed by infectivity tests on the one hand, and antigens as demonstrated by serological means on the other. This gap in knowledge will be considered more fully when we are dealing with the immunological implications of the carrier state.

Are infections of mice with LCM virus latent? Persistence of virus is well known in mice infected before or soon after birth. Pathological lesions are, however, not fully absent (see below). The findings in mice are inevitably confounded with the complexity of the responses of an animal. Persistent infections of mouse cells *in vitro* may be regarded as useful models to overcome this difficulty. Several reports have appeared describing observations on lymph node, embryo, and strain L cell cultures (Traub, 1962; Traub and Kesting, 1963; Benson, Magnusson, and Hotchin, 1961; Wagner and Snyder, 1962). The coexistence of LCM virus with L cells was more fully explored in a study in which monolayer cultures were infected with the WE strain virus and the multiplication of cells and infectious virus was followed for more than 160 days (Lehmann-Grube, 1967b). Functional and morphological impairment of cells was never observed after primary infection, although

cytopathology as well as retardation of the rate of multiplication became apparent during the first subculture. Very soon, however, the cells resumed their normal cultural characteristics, and if it had not been for their continuous release of infectious virus, they could not have been distinguished from the uninfected controls. While interference with the homologous WE strain or other LCM viruses was marked, interference with vesicular stomatitis virus could not be demonstrated, nor was interferon released into the medium.

This characterization of the chronic infection of L cells with the LCM virus was confirmed and extended with the E-350 strain of Armstrong (Lehmann-Grube, Slenczka, and Tees, 1969). Serial cultivation of virus-infected cells was easily accomplished (Fig. 4). Again, cells were damaged during early passages but quickly recovered to become indistinguishable morphologically, by their rate of multiplication, and their cloning efficiency from normal controls. Nevertheless, they produced large amounts of infectious virus and were stainable by conjugated antibody (Fig. 5). Thus, these cells were latently infected as defined by Andrewes (1958).

In most clones which were derived from single cells under conditions which definitely excluded spread of virus, each cell contained LCM virus-specific antigen. As with WE virus-infected L cells, homologous interference was marked, whereas heterologous interference could not be demonstrated. Treatment of the cells for a long time with LCM virus-neutralizing antibody slowly decreased the proportion of antigen-containing cells. Since an inhibitory action of antibody on infected cells could be excluded experimentally, it is likely that the persistent infection of L cells is perpetuated by horizontal spread in conjunction with vertical transfer from parent to daughter cells.

In a review on persistent viral infections of animal cell cultures, Walker (1964) defined four types. These were: (1) infections of cells partially resistant to the virus; (2) infections of susceptible cells protected by antibodies in the medium; (3) infections of susceptible cells protected by interference and interferon; (4) Regulated infections were characterized as follows: (a) Antibodies or other antiviral factors are not needed for maintaining an equilibrium between multiplying virus and cell viability nor is infectivity eliminated by antiserum. (b) Homologous interference is marked; heterologous interference is minimal or absent. (c) The virus multiplies in many or in all cells; single cells divide and grow into colonies, most of which are infected. A comparison of these criteria with our experimental results shows that LCM virus-L cell cultures belong to the category of regulated infections.

Fig. 5 Immunofluorescing antigen in L cells, persistently infected with LCM virus, strain E-350. Usually, antigen is found in the cytoplasm in the form of finely dispersed granules (upper part). In an occasional cell larger inclusion-like deposits are seen (lower part) (kindly supplied by Dr W. Slenczka, Marburg/Lahn).

The L cell line stems from a mouse, and though it is permanent and transformed, there is no cogent reason why knowledge gained *in vitro* should not be applied to the animal. Hence it is proposed that the infection of cells *in situ* is likewise self-regulated, ie, nonpathogenic, and that the signs which develop in neonatally as well as congenitally LCM virus-infected mice are due solely to some indirect effects of the virus on the host. As with mice infected when adult, disease in a carrier is probably also of an immunological nature. In order to demonstrate the absence of direct pathological effects of the multiplying virus on mouse cells *in vivo*, an experiment was performed which was based on the assumption that no immunological phenomena would interfere during the first few days of life in neonatally infected

Fig. 6 Growth of mice (as determined by weight measurements) infected with LCM virus at age less than 24 hours. Differently shaped symbols denote different litters, and bars refer to infectious virus (mouse ID₅₀) per gram of tissues (Lehmann-Grube, 1971).

mice (Lehmann-Grube, 1971). Litters each of eight stock albino mice were inoculated intraperitoneally, within 24 hours after birth, with 10 000 ID₅₀ (mouse) of LCM virus (strains E-350 or WE) and their growth was followed by recording weights at short intervals. As Figure 6 shows, the infection was without effect on the growth rates for at least five days, even though the virus multiplied to very high concentrations and reached titres of nearly 10¹⁰ ID₅₀ (mouse) per gram of tissues. The visual impression is confirmed by a mathematical analysis of the data. The regression coefficients for weight (gram) on time after infection (hour) were estimated as 0.017, 0.019, and 0.015 for WE-, E-350-, and sham-inoculated mice, respectively.

Having shown that the virus does not noticeably harm the mouse cell in which it multiplies, we may ask why is the infection not terminated by the host's immune mechanisms? For many years this question seemed to be satisfactorily answered by supposing

that mice are incapable of eliminating the agent because of a state of acquired immunological tolerance (Burnet and Fenner, 1949; Volkert and Larsen, 1965a). More recently, this view has been challenged by Oldstone and Dixon (1967, 1968, and 1969) on the grounds that persistently infected mice are capable of responding immunologically to viral antigens. Before entering into a discussion of this rather controversial issue, let us briefly summarize the knowledge of the immunological events in the mouse which follow the introduction of the virus.

After intracerebral infection of mature adult mice an illness which runs a characteristic course and usually leads to death develops in most animals; after peripheral infection few mice die. It is generally agreed that disease in an adult mouse infected with the LCM virus is due to an allergy directed against virus-induced new antigens (Hotchin, 1962; Lehmann-Grube, 1971).

Fig. 7 Neutralizing and complement-fixing antibodies in mice, infected with LCM virus, strain WE (Lehmann-Grube and Slenczka, 1971).

Whatever the route of inoculation, surviving animals rapidly develop protection against subsequent intracerebral challenge. Some resistance can be demonstrated as early as three days after a subcutaneous or intraperitoneal infection, and by day eight all mice withstand an otherwise lethal dose (Traub, 1936c; Lyon, 1940; Haas, 1954; Rowe, 1954; Seamer, Barlow, Gledhill, and Hotchin, 1963). At about the same time complement-fixing (CF) antibody appears. It becomes detectable around the eighth day and soon reaches moderate titres of 64 to 128 as is shown in Fig. 7 (Traub and Schäfer, 1939; Smadel and Wall, 1940; Weigand and Hotchin, 1961; Volkert, Larsen, and Pfau, 1964; Larsen, 1968; 1969b; Lewis and Clayton, 1969). By way of contrast, neutralizing antibody does not appear till the fourth week after infection, and the titres increase slowly reaching maximum values between 50 and 100 days (Fig. 7). Elimination of virus is likewise a slow process and viraemia may remain detectable for a long time. A few mice seem to retain traces of infectivity for months and presumably for life (Traub, 1938; Haas, 1954; Rowe, 1954; Traub, 1961; Lehmann-Grube, 1964a; Volkert, 1964; Volkert and Lundstedt, 1968; Larsen, 1969c). Although some neutralizing antibody can be detected in mice which have survived infection, elimination of the virus does not appear to be mediated by circulating antibody and is probably accomplished by cellular mechanisms. This conclusion was reached by Traub (1936c) many years ago. Since then numerous observations have been made which are in support of this view, and today there exists little doubt that delayed type hypersensitivity or related mechanisms terminate the infectious process (Lehmann-Grube, 1971).

What happens when the virus enters an immature ie, unborn or newborn mouse? Since differences between neonatal and congenital carriers are quantitative only, both will be treated together. The virus multiplies to high concentrations which are then maintained, usually for life. A few mice inoculated right after birth and found to be carriers have been later found to be free of virus (Lehmann-Grube, 1964b) but this does not appear to happen with congenital carriers (Lehmann-Grube, to be published). In addition to showing infectivity, all tissues contain CF antigen (Traub, 1961). As a result of a cooperative study conducted with my colleague, Dr H. Gschwender, it has been shown that most, if not all, antigens with CF activity are initially bound to membranous structures of the cells. Complement-fixing antigen can, however, be readily solubilized from infected cells and tissues and remains in the liquid phase after centrifugation at 120 000 g; in aging cell cultures it is set free spontaneously. The important question as to whether CF antigen circulates in the persistently infected mouse, either complexed to antibody or free, cannot at present be answered.

Virus-induced new antigens can also be demonstrated by transplantation, thus skin from neonatal or congenital carriers transferred to virus-free syngeneic recipients is in many instances rejected with complete or partial destruction of the graft in a manner closely resembling a homograft reaction (Holtermann and Majde, 1969; 1971; Lehmann-Grube, 1971).

As we have already seen, neonatal carriers develop normally during the first five days of life. They then pass through a phase of retardation which depends on the strain of the mouse and of the virus and this

leads either to severe runting with death or outwardly complete recovery. When approximately 1 year old, a disease may develop which is characterized by ruffled fur, blepharitis, and a hunched posture, followed by degenerative changes of eyes and skin and general wasting with early death (Traub, 1938; Hotchin, 1962; Hotchin, Benson, and Collins, 1963; Hotchin and Collins, 1964; Hotchin, 1965; Oldstone and Dixon, 1969). The severity of this 'late-onset disease' varies considerably in different strains of neonatally infected mice and is absent in some virus-mouse strain combinations (Hotchin and Collins, 1964; Volkert *et al*, 1964; Mims, 1966; Oldstone and Dixon, 1968; 1969; Oldstone, Habel, and Dixon, 1969); nevertheless histology shows that all neonatal carriers are affected to a greater or lesser extent (Hotchin *et al*, 1963; Collins and Hotchin, 1964; Hotchin and Collins, 1964; Baker and Hotchin, 1967; Oldstone and Dixon, 1969). With congenital carriers the situation is not as clear. At first sight these animals appear to be quite healthy but closer observation reveals certain abnormalities. Thus Mims (1970), who has followed the fate of congenital carrier mice for 600 days, found that birth weights were normal but litter sizes were reduced; again carriers grew slower and remained lighter. Throughout life, mortality in these mice was higher than in controls and these definite, if slight, signs of retardation are reflected by pathological alterations in many organs which were first described by Traub more than 30 years ago (Traub, 1936c; Mims, 1970; Oldstone and Dixon, 1970b). Thus, while there are differences in severity of late-onset disease between neonatal and congenital carriers, neither may be regarded as a true example of latency which, as will be recalled, has been defined as persistent and inapparent.

Before we can discuss the mechanism that prevents the mouse from terminating the infection immunologically—which under other circumstances it is undoubtedly capable of doing—we must scrutinize the antigens which are involved. In 1939 Smadel, Baird, and Wall (1939) presented some evidence which indicated that complement-fixing antigens and virus surface are immunologically distinct. In contrast, preliminary data of Barlow and Mustico (1965, 1966) seemed to prove the contrary. Consequently, in a recent review on the LCM virus, I wrote as follows (Lehmann-Grube, 1971): 'Since the antigens which are responsible for immunological responsiveness and tolerance have not yet been identified, it is not surprising that we do not fully understand the nature of the immune processes involved. The experimental data which have direct bearing on this problem have all been obtained with the whole virus. The following

discussion is therefore presented with the reservation that the identification with the virus of the antigen "X", which is responsible for the immunological conflict, is so far not much more than a working hypothesis. The situation is further complicated by the fact that the relationship between virus and s-antigen is virtually unknown.... In particular, these may differ antigenically, and since all tissues produce the soluble antigen, the data obtained with the virus may apply to the soluble component as well. In order to facilitate our discussion, we shall make the most simple assumption, namely, that the s-antigen is part of the virus structure and antigenically not different from it.' The discussion then concentrates on the apparent discrepancy in that antibody is detected in mice unable to eliminate the virus, and since there is good reason to believe that virus elimination is executed solely by cellular immune processes, much is made of the separation of cell-mediated and humoral immunity in these animals.

In recent months, we have begun to reinvestigate the relationship between virion and LCM virus-specific CF antigens. Although it is too early to draw final conclusions, it seems likely that 'the most simple assumption' was wrong; intact virus and CF antigen appear to be immunologically distinct. This conclusion is based on three lines of evidence: (1) concentrated and partially purified virus fixes little or no complement in the presence of an antiserum which, together with the appropriate antigen, has a high binding capacity; (2) concentrated and partially purified CF antigen with high specific titre, prepared from infected L cells or guinea-pig organs, has no blocking effect on the neutralization of infectivity by rabbit immune serum; (3) guinea-pigs, repeatedly inoculated with concentrated and partially purified CF antigen, are not protected at all against later challenge with infectious virus (Lehmann-Grube and Gschwender, to be published). Furthermore, immunological diversity explains the difference in development of CF antibody and elimination of virus, as observed under certain experimental conditions, much better than has been previously possible (Hannover Larsen, 1969a) (Fig. 8). Presumably, this phenomenon simply denotes different responsiveness of the mouse towards unrelated antigens.

For many years it seemed established that LCM virus carrier mice are immunologically tolerant in respect of the virus. Though responding in a normal fashion to numerous other antigens, such as eastern equine encephalomyelitis virus (Traub, 1961), St Louis encephalitis and influenza viruses (Haas, 1954), sheep red cells (Mims and Wainwright, 1968; Oldstone and Dixon, 1971a; 1971d), allogeneic transplants (Volkert and Larsen, 1965b; Lehmann-Grube and Niemeyer, 1971; Holtermann and

Majde, 1971; Suzuki and Hotchin, 1971; Oldstone and Dixon, 1971a and d), and keyhole limpet haemocyanin (Oldstone and Dixon, 1971a and d), these animals were thought not to develop immunity to LCM viral antigens. This assumption still reflects current knowledge as far as cellular immunity is concerned, although a few experimental results (Lundstedt, 1969; Oldstone and Dixon, 1971a) have been interpreted to prove the opposite (Oldstone, 1971; Oldstone and Dixon, 1971a). By way of contrast, humoral immunity can be detected more convincingly in these mice. As far back as 1939, Traub and Schäfer (1939) had found CF antibody in a few carriers. This long-neglected report was recently confirmed and extended by Oldstone and Dixon (1967, 1968, and 1969). In the kidneys of neonatal carriers, accumulations of host γ -globulin as well as the third component of complement were demonstrated by immunofluorescence and traces of these were found as early as 10 days after infection. In acid eluates from such organs low concentrations of LCM virus-specific CF antibody were detected which increased with the age of the animal. Half the γ -globulin in the eluates could be absorbed with LCM virus-infected monkey kidney cells but not with control cells. Labelled guinea-pig complement was eliminated more rapidly from carriers than from normal mice. Later, essentially the same observations were reported in congenital carriers (Oldstone and Dixon, 1970b). Antibody was also detected in neonatal carriers by means of the immunofluorescence technique from about two months after infection (Benson and Hotchin, 1969). No doubt, these findings show that LCM virus carrier mice can make antibodies against some virus-specific antigens. They do not, however, prove that these animals can respond immunologically to the surface of the virion, and only such a response could play a role in the elimination of the virus. Here our knowledge is limited. According to Oldstone and Dixon (1969; 1970b), infectivity of serum from carrier mice is lost by precipitation with antisera against mouse IgG and mouse complement (C3) but not with anti-IgA or IgM antisera. In a similar situation, namely, the persisting infection of murine sarcoma virus in BALB/c mice, Hirsch, Allison, and Harvey (1969) found that anti-IgM but not anti-IgG or anti-IgA neutralized the circulating virus. In general, this observation has been confirmed by myself (details to be published): precipitation of serum from carrier mice with antiserum prepared in goats against mouse immune globulin removed infectious virus, whereas cell culture-grown virus suspended in normal mouse serum remained unaffected by this treatment. However, rather surprisingly, attempts to detect antibody bound to carrier virus by neutraliz-

ation with antibody against mouse globulins have failed. In spite of the use of different sera and the variation of experimental conditions, not a trace of sensitization of virus from persistently infected mice could be demonstrated. A typical experiment of this kind is summarized in Table I. Similarly, Suzuki and

Sera		Diluted	Titre (LD ₅₀ /ml)
Produced	By		
Against	By		
IgG + IgM	Melpar	—	5.02
IgG + IgM	Melpar	1:10	4.40
Ig	Hyland	—	4.52
Ig	Hyland	1:10	4.65
—	Control ¹	—	4.85
—	Control	1:10	4.74
—	Diluent ²	—	4.30

Table I. Effect of anti-mouse-immune-globulin antiserum (goat) on infectious titre of LCM virus from carrier mice

¹ Assayed intracerebrally in mice.

² Normal goat serum.

³ Hanks' BSS plus 1% inactivated calf serum.

Hotchin (1971) could not demonstrate neutralization of virus from the serum of mice, made carriers by infection after weaning with high doses of virus, by anti-mouse γ -globulin antiserum, although antibody, assayed by immunofluorescence, was present in these animals. Nevertheless, it now seems clear that both virus and CF antigen are not eliminated from carrier mice, although immune responses are not entirely absent as evidenced by the appearance of antibodies.

Elimination of the LCM virus is probably caused entirely by mechanisms akin to delayed type hypersensitivity. The assumption could be made that, in a carrier mouse, immunological tolerance consists of two separable components, one concerned with circulating antibodies, the other with cellular immunity, as has been found to be the case with other antigens (Turk and Humphrey, 1961; Battisto and Chase, 1965; Borel, Fauconnet, and Miescher, 1966). Notwithstanding this speculation, it should be stressed that immunological tolerance is not an all-or-none phenomenon but rather an 'impressed diminution in immunologic responses below those which normally occur after known antigenic excitation' (Chase, 1959), and it is not at all contradicted by the demonstration of traces of specific antibody. A further consideration, expressed previously (Lehmann-Grube, 1971), may be repeated here. 'Antibody concentrations in LCM virus-infected mice occur at two levels (if we disregard in this context the high titres reached after adoptive immunization of carrier mice), and both the minute

Fig. 8 Independence of viraemia and complement-fixing antibody in mice, infected with LCM virus, strain Traub (Hannover Larsen, 1969a).

amounts which are just barely detectable in mice infected neonatally as well as the comparatively high concentrations, such as are found after non-lethal infections of adult mice, may be associated with virus concentrations characteristic of carrier mice. If, as Oldstone and Dixon (1969) suggested, the low concentration of antibody in carrier mice were a peripheral phenomenon in the sense that 'this antibody cannot be detected free in the circulation because of an excess of antigen or virus', then normal antibody concentrations in carrier mice could only be due to less efficient masking, representing merely the other extreme of the same phenomenon. If this were true, carrier mice with intermediate levels of antibody concentration should be found more frequently than either or both of the two extremes. This, however, is definitely not the case. Although conversions take place, as may be seen in Fig. 8, intermediate grades of antibody concentrations are not known to occur and it appears reasonable to assume that true differences exist.' Similar thoughts have been expressed by Volkert and his colleagues (Volkert and Larsen, 1965a; Volkert and Lundstedt, 1971), and we may conclude that present knowledge does not compel us to discard

the hypothesis of virus-specific immunological tolerance as a basis for the persistence of virus.

Until now, the term 'immunological tolerance' has been used strictly in an operational sense, and the question whether this phenomenon is caused by central failure—as, for instance, by physical or functional elimination of the competent cells—or by antibody which blocks either effector or target cells (Hellström and Hellström, 1970; Mitchison, 1971) has not entered our discussion. In the persistent infection of the mouse with LCM virus there are two aspects of hypo- or unresponsiveness. One concerns the virion surface: virus is not eliminated in spite of attached antibody, and it is tempting to speculate what role this antibody may play in protecting the virion against the action of sensitized lymphocytes. This antibody does not appear to occupy sites which are essential for infectivity and, hence, we have to make the additional assumption that the viral antigens which induce cellular and humoral responses are different. Specific hyporesponsiveness in carrier mice also exists in respect of CF antigen: as in the case of the virus, CF antigen is not eliminated and antibody is formed in trace amounts. Again, little can be said of the underlying mechanism, although in this case our attempts to detect blocking activities in sera from carrier mice have failed (to be published).

Recently, Hotchin (1971a) has summarized his view on the mechanism by which specific immunological unresponsiveness in LCM virus carrier mice is induced and maintained. He proposes that the virus in the mouse consists of two types of virions (Hotchin, Kinch, and Benson, 1971), both of which infect lymphoid cells, preferentially those of thymic origin. If the infecting virus is of the 'L type', the lymphocyte is thought to be destroyed by contact with other immune cells sensitized to new virus-induced antigens. If, on the other hand, the lymphoid cell is infected by the 'I type' variant of LCM virus, new virus-induced antigens do not appear and infection by the potentially harmful 'L type' virion is prevented by interference. Furthermore, acquisition of the viral genome does not permit these 'shutdown cells' to respond immunologically to LCM virus-specific antigens. Thus, 'tolerance is . . . seen as a dual phenomenon, resulting in part from immune paralysis and in part from infestation of the host with a large population of virus-infected but "shutdown" cells'.

For various reasons, this hypothesis does not satisfy. First, it would demand that some immunodepression is found throughout the life of a carrier mouse, and this is definitely not the case. Though depression of immunological reactivity follows the infection of newborn and adult mice with LCM virus (Mims and Wainwright, 1968; Lehmann-

Grube, Niemeyer, and Löhler, 1972), this deficiency is short and lasts—when tested for by the ability to mount a homograft response—a few days only; later, carrier mice readily respond to all sorts of antigens (see above). Furthermore, immunodepression occurs independently of apparent pathological alterations in lymphoid organs (Lehmann-Grube *et al.*, 1972). Secondly, Lehmann-Grube and Raff (1971) have shown experimentally that LCM virus does not preferentially destroy thymus-derived lymphocytes; the proportions of theta antigen-bearing cells of spleens were not reduced in mice at various times after the intracerebral infection with LCM virus. On the contrary, the majority of assays proved that their numbers were relatively increased. According to Oldstone and Dixon (1971d), theta-bearing cells are not reduced in lymph nodes or spleens of carriers; nor are the numbers of circulating lymphocytes depressed in these mice. Thirdly, untreated peripheral mouse lymphocytes were found to resist infection with LCM virus *in vitro*, although after stimulation with phytohaemagglutinin (PHA) these cells readily produced infectious progeny (Schwenk and Lehmann-Grube, 1971). Furthermore, experiments performed by myself (details to be published) have given no hint that lymphocytes in LCM virus-infected mice contain or produce virus. Thus, mouse lymphocytes do not appear to participate in the

infectious process, and it is not at all surprising to find that blood lymphocytes from carrier mice respond quite normally to the stimulating effects of PHA (Schwenk, Slenczka, and Lehmann-Grube, 1971).

We may now ask, What is the nature of the pathological alterations which are found in neonatal as well as congenital carriers? For reasons already discussed, direct effects of the virus on the infected cells seem to be an unlikely mechanism. Damage of tissues in these mice as a result of an immunological host response against antigens which have appeared as a consequence of the virus infection is an attractive explanation. The details are obscure but one widely accepted interpretation is based on the work of Dixon, Feldman, and Vazquez (1961) who produced chronic glomerulonephritis in rabbits by prolonged intravenous applications of various heterologous proteins. Accordingly, circulating virus-antibody complexes are thought to lodge in the organs of carrier mice, thus causing the observed tissue damage (Oldstone and Dixon, 1969, 1970a, 1970b, and 1971b; Oldstone, 1971). The quality and quantity of complexes formed in the blood of carriers by infectious virus or CF antigen and their respective antibodies are entirely unknown. In mice carrying the lactic dehydrogenase virus—which circulates complexed with antibody (Notkins, Mahar, Scheele, and Goffman, 1966)—clinical disease is absent and

Fig. 9 Immunology of the persistent infection of the mouse with lymphocytic choriomeningitis virus.

histological changes in the kidneys are minimal, although large amounts of host Ig, presumably complexed with viral antigen, are deposited (Oldstone and Dixon, 1971c; Porter and Porter, 1971). For these reasons, I agree with Hotchin (1971b) who advocates 'a cautious interpretation' of the complexes found in kidneys from carrier mice.

While circulating antigen-antibody complexes and the role they may play in the pathogenesis of the disease in LCM virus carrier mice are of unknown significance, no doubt exists that CF antigen, as well as infectious virus, forms part of many cells in all tissues. Thus, traces of antibodies are produced against heterologous cellular antigens which have appeared in the mouse very early—possibly on day zero of its existence—and which may be assumed to have been integrated into the antigenic make-up during immunological immaturity of the host and are thereafter recognized as self rather than foreign. Taking this view, pathological alterations in LCM virus carrier mice are, at least formally, related with autoimmune phenomena and are comparable to a great number of pathological disorders in animals and man caused or maintained by immunity against self components. The relationship with autoimmunity may be even closer than that. It is quite conceivable that immune responses are not directed against viral antigens but rather against the host's own tissues which have taken on autoantigenicity because of alteration, induction, or demasking due to the viral infection, as has been proposed to occur with LCM virus by Hotchin (1962, 1971b) and with other lipid-containing RNA viruses by Drzeniek and Rott (1969). Alternatively, one could think of a mechanism in which viral and cellular components cooperate antigenically in a manner analogous to the hapten-carrier cooperation, thus inducing autoimmunity (Allison, Denman, and Barnes, 1971).

The immunology of the persistent infection of the mouse with the LCM virus, as expounded on the previous pages, is summarized in Figure 9. This scheme is not thought to provide final answers to our questions. It is nothing more than a working hypothesis designed to elucidate the complexity of the interaction between LCM virus and mouse, thus being of assistance in further experimentation.

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