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# Viruses: immunosuppressive effects

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Many virus infections in man and other species are accompanied by immunosuppression. This is clearly important in terms of the susceptibility of the host to secondary infections. The immunosuppression may also aid and abet the growth and persistence of viruses. An unresolved issue is the extent to which the extent of this immunosuppression is determined by the virulence of the infecting virus or resistance factors in the host, and particularly by factors that are genetically determined. The mechanisms of viral immunosuppression are indirect and direct. Indirect mechanisms such as interferon production and suppressor cells induced by infection undoubtedly contribute to viral immunosuppression in experimental models of virus infection. In man the direct inactivation of immunologically responsive lymphocytes seems to be the most important mechanism. Moreover, the persistence of viruses in human lymphocytes is being increasingly recognized.

## 1. Introduction

The immunosuppressive effects of virus infections are well recognized; a variety of cell-mediated and humoral immune responses may be affected. It is necessary to distinguish between specific interference with immunity to the invading virus and non-specific immunosuppression, and an important issue is to determine the extent to which immunosuppression induced by a given virus contributes to its virulence in acute infections. A related problem is to explore the link between specific immunosuppression and viral persistence. Non-specific immunosuppression may render the host vulnerable to secondary infection by environmental agents and to reactivation of latent viruses.

## 2. Specific suppression of antiviral immunity

Several forms of microbial infection provide good precedents for the idea that specific immunosuppression of antiviral immunity helps the invading virus to evade host immune responses. For example, different strains of trypanosome suppress the immune responses of the host to varying extents and their virulence can be correlated with the extent of this immunosuppression (Sacks *et al.* 1980). The IgM antibody response is particularly susceptible. Similarly, viral immunosuppression may have striking consequences for the infected host. For example, several species of parasitoid wasps of the family Ichneumonidae carry a virus in the calyx cells adjacent to the oviduct. This virus suppresses the immune responses of the caterpillar host so that wasp eggs are not rejected after their injection into the caterpillar haemocoele (Edson *et al.* 1981). There are also instances of virus infections that interfere with specific antiviral immunity. Mice lethally infected with virulent strains of street rabies virus fail to develop specific cytotoxic T lymphocyte responses to virus-infected target cells; in contrast, mice



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surviving infection by attenuated rabies virus generate a brisk response of this kind (Wiktor *et al.* 1977). Lymphocytic choriomeningitis (LCM) virus produces a variety of disease syndromes in mice depending upon the strain of virus, the route and dose of infection, and the age at which the mice are infected. Infected mice may succumb to acute encephalitis or survive with chronic immunopathological disorders. Cytotoxic T lymphocytes specifically immune to LCM-infected cells play a dual role in eliminating virus and initiating the immunopathological consequences of acute and chronic infection. Dunlop & Blanden (1977) have shown that the generation of a virus-specific cytotoxic reaction is suppressed both *in vitro* and *in vivo* by exposing the progenitor cells to high levels of infectious virus. They postulated that persistent infection results from the deletion of T lymphocytes arising from the lymphocyte clone specifically sensitized to this virus. Extending these observations, Thomsen *et al.* (1982) have analysed the specific depression of cytotoxic T lymphocyte responses in mice with general immunosuppression induced by infection with LCM virus. They attributed the marked depression of this response to viral interference with T-cell maturation during the early stages of infection.

There is also evidence that viruses administered by unusual routes interfere with antiviral immunity. For example, the intravenous injection of herpes simplex virus in mice ablates delayed-type hypersensitivity reactions to the virus. However, such mice still develop protective immunity as shown by the rapid disappearance of infectious virus (Nash *et al.* 1983). There is no evidence as yet that the selective depression of such antiviral responses achieved in this way increases viral virulence.

There are few clear-cut examples of specific immunosuppression in viral infections of man. However, such reactions may be masked by the wide array of non-specific responses which the infected human host is able to deploy during the initial stages of infection. Infection by Epstein–Barr (EB) virus illustrates this point. Primary infection by EB virus is commonly associated with the self-limited lymphoproliferative disease infectious mononucleosis. The major part of the characteristic atypical lymphocytosis consists of activated mononuclear cells participating in the host response to B lymphocytes infected with EBV (Denman & Pelton 1974; Svedmyr & Jondal 1975). This point was established by experiments *in vitro* in which the lymphocytes of patients with infectious mononucleosis were shown to be cytotoxic for lymphoblastoid cell lines transformed by EB virus. It was originally thought that the reaction was directed at antigens related to EB virus; however, it has since become apparent that the cytotoxic cells do not belong to a single population. At least some of the cytotoxicity is mediated by T lymphocytes that kill lymphoblastoid cell lines lacking the EB virus genome (Patel *et al.* 1982). In addition natural killer (NK) cells also contribute to the cytotoxicity for transformed cell lines. Other hitherto unsuspected host defence mechanisms may also play a part; thus Griffith *et al.* (1982) have shown that immediate hypersensitivity reactions are induced by experimental infection with cytomegalovirus (CMV). There is no doubt that a specific T lymphocyte response to lymphoblastoid cells infected by EB virus evolves during the course of infection and that this response is governed by the same requirement for histocompatibility between target cells and cytotoxic lymphocytes as that noted with other viruses (Wallace *et al.* 1982). Moreover, an efficient T-cell reaction against cells infected with EB virus may be necessary for limiting subsequent virus reactivation (Gaston *et al.* 1982). The intriguing issue is the extent to which the generation of specific immunity to EB virus may be impaired during primary infection. Because the host is protected by non-specific immunity in the form of NK cells and possibly other mechanisms, this immunosuppression would rarely be clinically



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apparent, and specific immunity would eventually be established in the vast majority of infected individuals. In contrast, clinical observation shows that patients who develop unusual manifestations of EB virus infection have persistent, readily detectable defects in specific immunity to this virus. Patients with the X-linked lymphoproliferative syndrome (Duncan's disease) have received particular attention (Purtilo *et al.* 1979). Two-thirds of the 100 males with this disorder so far studied have died from uncontrolled B lymphocyte proliferation during the initial attack of infectious mononucleosis, whereas most of the survivors have developed hypogammaglobulinaemia or lymphomas. Patients with this syndrome have defective immune responses to EBV virus in the form of subnormal responses to the EB virus-coded nuclear antigen (EBNA) and poor *in vitro* cytotoxic T cell responses to autologous, EB virus infected lymphoblastoid cell lines (Harada *et al.* 1982). Whereas B lymphocytes from normal donors produce immunoglobulin *in vitro* after stimulation with appropriate mitogens, B lymphocytes from these patients produce subnormal or undetectable amounts of immunoglobulin (Lindsten *et al.* 1982). These defects may be genetically determined in the sense that individuals with the disorder are incapable of mounting an effective anti-viral response. Alternatively, virus growth may result in selective immunosuppression in those who are genetically susceptible to this complication of the primary infection.

### 3. Non-specific immunosuppression

The clinical relevance of non-specific immunosuppression is more clear cut. Thus the mortality associated with influenza virus infections is largely attributable to secondary bacterial infection resulting from the disruption of host defences (Couch 1981). Similarly, the bacterial and viral infections complicating endemic measles infections follow the immunosuppression induced by this virus (Whittle *et al.* 1978).

There is good evidence in experimental infections that the virulence of certain viruses can be correlated with the extent to which these induce non-specific immunosuppression. For example, murine cytomegalovirus (CMV) suppresses the blastogenic response *in vitro* to non-specific mitogens of spleen cells from infected mice (Allan *et al.* 1982). However, the immunosuppression induced in genetically susceptible BALB/c mice exceeded that observed in genetically resistance BALB/K mice even though virus infectivity titres were similar in tissues assayed from both strains. There is, however, little evidence that the immunosuppressive effects of virus infections in man are related to the virulence of the infecting strain.

### 4. Mechanisms of viral immunosuppression: general considerations

Several possible mechanisms of viral immunosuppression must be considered. Viruses may infect lymphocytes and depress their function in a variety of ways including inhibition of protein synthesis and interference with membrane receptors. Alternatively viral infection of cells other than lymphocytes may be immunosuppressive. Monocytes, macrophages and dendritic cells ('veiled' cells) play a crucial role in presenting antigen to T lymphocytes in immunogenic form. Since it has been shown that many viruses grow in blood monocytes and macrophages from different sources, the contribution of these cells to immune responses may be thereby affected. Several viruses activate suppressor mechanisms among which suppressor T cells have received particular attention; this activation could result in specific or general immunosuppression.



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Again, infections stimulate the production of soluble factors that influence immune reactions. The interferons have been the most closely studied and it is clear that these may enhance or suppress immune reactions depending on the experimental conditions. Finally, non-specific activation of B lymphocytes by viral glycoproteins (Goodman-Snitkoff *et al.* 1981) may restrict the host's ability to respond to new antigens in a manner analogous to that observed in some bacterial infections (Grooten *et al.* 1980).

TABLE 1. PRODUCTIVE VIRUS GROWTH IN HUMAN LYMPHOCYTES

| virus | susceptible cells† | reference‡ |
|---|---|---|
| | (*a*) *DNA viruses* | |
| adenovirus | lymphocytes; B cell lines | Andiman & Miller (1982) |
| herpes simplex (types 1 and 2) | T and B lymphocytes | Kirchner *et al.* (1977) |
| cytomegalovirus | monocytes | Rinaldo *et al.* (1979) |
| | B and T cell lines | Tocci & St Jeor (1979) |
| Epstein–Barr virus | B lymphocytes | Epstein (1982) |
| vaccinia | lymphocytes | Miller & Enders (1968) |
| | (*b*) *RNA viruses* | |
| poliovirus | lymphocytes | Willems *et al.* (1970) |
| parainfluenza | lymphocytes | Verini & Lief (1979) |
| measles | T and B lymphocytes | Joseph *et al.* (1975) |
| Newcastle disease virus | lymphocytes | Duc Nguyen & Henle (1966) |
| mumps | T lymphocytes | Fleischer & Kreth (1982) |
| rubella | lymphocytes | Van der Logt *et al.* (1980) |
| alphaviruses | monocytes | Levitt *et al.* (1979) |
| dengue | monocyes; monocyte lines | Brandt *et al.* (1982) |

† Peripheral blood in all instances.
‡ Selected recent publications.

## 5. VIRUS GROWTH IN LYMPHOID CELLS

Many viruses grow in lymphocytes or other cells of the lymphoid system as an obligatory stage in the pathogenesis of infection. This discussion primarily concerns virus infections of man and experimental infection of human lymphoid cells. The relation between viruses and mononuclear cells can be explored by seeking evidence *in vivo* of infection by using conventional methods for isolating virus or more sophisticated techniques for detecting viral products. Alternatively it is possible to infect lymphoid cells *in vitro* after appropriate immunological stimulation. Several viruses have been recovered from human blood lymphocytes, often at considerable intervals after the original infection. Rubella virus is a remarkable example of this persistence: Chantler *et al.* (1982) have isolated rubella virus from blood lymphocytes of patients with post-rubella arthritis 6 years after the primary infection. It is noteworthy that no generalized immunodeficiency was detected in these individuals. However, the atypical arthritis noted in these and other patients (Grahame *et al.* 1983) suggests that the rubella infection followed an unusual course, possibly resulting from a specific and so far uncharacterized defect in immunity to rubella virus. There seems little doubt that their arthritis resulted from rubella infection.

Several viruses have been shown to infect stimulated human lymphocytes and monocyte–macrophages (table 1). The effects on immunological function have been examined mainly in terms of responses to non-specific mitogens, and such reactions are indeed suppressed by many viruses that grow in lymphoid cells. However, responses of this kind bear little relation to selective responses *in vivo* induced by antigens, and experiments with the use of these systems



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do not take into account the complex interactions between different lymphocyte populations. The introduction of techniques for inducing specific antibody production by human lymphocytes *in vitro* has allowed the immunosuppressive effects of virus infection to be analysed in detail (Platts-Mills & Ishizaka 1975; Callard 1979). These methods show that antibody synthesis *in vitro* depends upon the interaction of inducer T lymphocytes, accessory cells and B

TABLE 2. CULTURED HUMAN LYMPHOCYTES; LIMITED SUSCEPTIBILITY TO VIRUS INFECTION

| virus | cells | assay | percentage infected | reference |
|---|---|---|---|---|
| HSV type 1 | antigen-stimulated tonsil cells | ICA† | 0.08‡ | Pelton *et al.* (1977) |
| HSV ts mutants | antigen-stimulated tonsil cells | ICA | 0.11‡ | Pelton *et al.* (1980) |
| CMV | lymphoblastoid cell line | ICA | 1.0–10.0 | Furukawa (1979) |
| EBV | blood lymphocytes | limiting dilution | 0.20 | Yarchoan *et al.* (1983) |
| measles | antigen-stimulated tonsil cells | ICA | 0.03 | Pelton *et al.* (1982) |

† ICA, infective centre assay.
‡ 24 hours after infection.

lymphocytes. Herpes simplex virus (HSV) for example, grows in mitogen-stimulated T and B lymphocytes (Kirchner *et al.* 1977). This virus suppresses specific *in vitro* antibody production to diphtheria toxoid by human tonsil cells (Pelton *et al.* 1977). However, in this system the virus grows predominantly in T lymphocytes and the immunosuppression results from virus growth in inducer T lymphocytes during the early stages of antibody induction. Virus added after the first 24 h of culture still replicates in susceptible cells but antibody responses are not affected. Similar observations have been made in cultured tonsil cells infected with measles virus. Like HSV, measles virus grows in mitogen-stimulated T and B lymphocytes (Joseph *et al.* 1975). The virus also suppresses antibody synthesis *in vitro* by its effect on inducer T cells, but again only during the early stages of the response (Pelton *et al.* 1982). Although virus grows in B lymphocytes, antibody synthesis and secretion are not affected.

Experiments with temperature-sensitive (ts) mutants of HSV show that the immunosuppressive effects of this virus can be correlated with the inhibition of protein synthesis in infected inducer T cells (Pelton *et al.* 1980). Only those temperature-sensitive mutants that inhibit protein synthesis suppress antibody synthesis.

It is noteworthy that lymphocytes in virus-infected cultures are not uniformly vulnerable to infection (table 2). Only a limited number of lymphocytes respond to antigen challenge and this restricts the number of cells initially susceptible. However, for less obvious reasons, there are other situations in which virus infects only a small proportion of lymphocytes, whether these are activated by immunological manoeuvres or by the virus directly. For example, only 0.2–1.0% of blood B lymphocytes are susceptible to transformation by EB virus (Yarchoan *et al.* 1983). Similarly, lymphoblastoid cell lines of different origin show different susceptibility to virus infections. Studies of a lymphoblastoid cell line that continued to support CMV replication showed that the number of infected cells never exceeded 10% of the total cell count (Furukawa 1979). The stability of the CMV genome also depends on the nature of the infected cell line, the number of viral DNA copies remaining constant in some lines and diminishing in other (Tocci & St Jeor 1979).

Some of the factors determining the susceptibility *in vitro* of lymphocytes to infection are known. These include the availability of virus receptors and the differentiation stage of cells



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such as B lymphocytes (Katz *et al.* 1981). Simply allowing neonatal human lymphocytes to age in culture confers permissiveness to HSV infection on these cells (Grogan *et al.* 1981). Immunological factors are also involved. Hammer *et al.* (1981) have shown that suppression of herpes simplex virus in a human T lymphoblastoid cell line is mediated by antiviral antibody, the virus reactivating once antibody is removed. Macrophages from a mouse strain that is genetically resistant to infection by mouse hepatitis virus become susceptible to infection if isolated from cortisone-treated donors, through an effect mediated by splenic lymphocytes (Taylor *et al.* 1981).

### 6. Viral persistence in lymphocytes

Many viruses undergo a complete replication cycle in lymphocytes with the production of infectious progency. However, some viruses produce latent infection of lymphocytes, or they only complete part of their growth cycle in these cells. Measles virus, for example, silently infects unstimulated lymphocytes; only occasional infected cells can be detected by infectious centre assay, immunofluorescence or electron microscopy (Lucas *et al.* 1978). Nevertheless latent virus is readily reactivated by exposing the infected lymphocytes to phytohaemagglutinin. Latent persistence of measles virus infection in lymphocytes has been attributed to the failure of these cells to cleave the virus fusion protein needed for productive infection (Fujinami & Oldstone 1981). Interferon produced in low concentrations may also account for latency because it is abrogated by exposing the cells to anti-interferon antibody (Jacobson & McFarland 1982). Most strains of influenza A and B virus also fail to infect lymphocytes productively. Nevertheless several virus-specific proteins including nucleoprotein and membrane protein were synthesized by lymphocytes infected with several strains of influenza A virus that failed to produce infectious virus (Brownson *et al.* 1979).

The clinical significance of virus persistence in lymphocytes has been repeatedly but inconclusively discussed in the context of chronic measles infection of the central nervous system (subacute sclerosing panencephalitis), demyelinating diseases and autoimmune diseases. It has been postulated that measles virus persistence in the lymphocytes of patients with subacute sclerosing panencephalitis is evidence of specific immunodeficiency in this disease despite normal conventional cell-mediated and humoral antibody responses to measles virus. Specific immunodeficiency to both HSV and CMV has also been implicated in patients with acquired immunodeficiency disease (Center for Disease Control 1982), and CMV RNA has been detected in the cells of the Kaposi's sarcoma frequently encountered in these patients (Fenoglio *et al.* 1982). Recently, too, Eglin *et al.* (1980) have detected RNA complementary to herpes simplex virus in blood lymphocytes of patients with Behcet's syndrome, a vasculitic disorder characterized by recurrent orogenital ulcers, uveitis, arthritis and encephalitis. However, the introduction of more sophisticated techniques for detecting viral genomes seems likely to reveal that persistent viral infection of lymphocytes is more common than had been imagined. For example, Peden *et al.* (1982) have used homologous DNA probes to detect fragments of herpes simplex virus DNA in apparently normal mammalian cells. The problem may therefore be to unravel the factors that lead to inappropriate viral reactivation in lymphocytes. There are some clues to the factors that might allow latent virus in lymphocytes to be reactivated. Thus Jordan & Mar (1982) have shown that murine CMV remains dormant in splenic B cells but becomes activated in spleen explants, particularly after secondary growth in macrophages. This observation suggests that a variety of immunological mechanisms keep latent viruses in check and that their abrogation may lead to inappropriate viral reactivation.



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### 7. Virus infection and accessory cells

Several viruses grow in monocytes or macrophages isolated from different sources. The ability of dengue virus to grow in human monocytes has been correlated with the virulence of different strains (Halstead *et al.* 1981) and this may be enhanced by cross-reacting antibodies between dengue virus and other flaviviruses (Brandt *et al.* 1982). In general the relations between virus infection of antigen-presenting accessory cells and immunosuppression remains speculative.

### 8. Suppressor cells and viral immunosuppression

Although there are several reports that activation of suppressor cells contributes at least in part to experimental viral immunosuppression, there is less evidence to this effect in viral infections of man. For example, suppressor cells do not account for the immunosuppression *in vitro* of specific antibody production induced by either HSV (Pelton *et al.* 1980) or measles virus (Pelton *et al.* 1982). However, there are reports that suppressor mechanisms operate during mononucleosis induced by both EB virus and CMV virus (Ho 1981; Carney & Hirsch 1981). This interpretation is partly based upon analyses of lymphocyte subpopulations by using monoclonal antibodies and which reveal an excess of cells with the phenoype of cytotoxic-suppressor cells. Other evidence stems from assays *in vitro* of lymphocyte responses to standard mitogens and their inhibition by autologous suppressor T lymphocytes or monocytes (Carney *et al.* 1983). The significance *in vivo* of these observations is uncertain; secondary opportunistic infections are rarely encountered in infectious mononucleosis and there is no evidence that abnormal suppressor activity contribute to the pathogenesis of atypical primary infections by EBV or CMV.

### 9. Interferons and immunosuppression

The immunoregulatory properties of the interferons have been extensively investigated (De Maeyer & De Maeyer-Guignard 1982). Interferons α and β, induced in most cell types by virus infection, and interferon-γ, a lymphokine produced by immune lymphocytes in response to virus-specific antigens, all influence both antibody and cell-mediated immune responses. Pure interferons α and β may enhance or suppress responses *in vitro* and *in vivo* depending on the time relation between antigen challenge and the administration of interferon, and also on the dose of interferon. Similar factors determine the effects of interferon-γ on immune responses with the added complication that the sources of interferon-γ used so far in such experiments are contaminated with other lymphokines. Although interferon undoubtedly limits virus growth it has been postulated that the accompanying immunosuppression may make virus persistence more likely (Merigan 1974). For example, interferon restricts viral replication in mice infected with CMV (Chong *et al.* 1983), but several aspects of lymphocyte function are suppressed in both acute and persistent infections by this agent. Spleen lymphocytes from infected mice produce both interferon-α and interferon-β after challenge *in vitro* with CMV (Kelsey *et al.* 1982), an observation suggesting at least one plausible mechanism for continued interferon production. Obviously it is difficult to dissociate the immunosuppressive effects of virus and interferons in such models. There is the same dilemma in human virus infections. Influenza and influenza-like illness, for example, are caused by viruses that variously depress immune responses (Roberts 1982) and also induce interferonaemia (Green *et al.* 1982). Most studies of effects of interferon on human lymphocytes have used non-specific stimulation of lymphocytes



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by mitogens *in vitro*. Thus interferon depresses the polyclonal production of Ig by B cells stimulated with pokeweed mitogen (Fleisher *et al.* 1982). More sophisticated systems reveal that interferon affects human lymphocyte function in a highly selective manner (table 3). Antibody synthesis to specific antigen dependent on inducer T lymphocytes is suppressed by interferon-α. In contrast, antibody production of the same specificity can be induced by polyclonal stimuli

TABLE 3. INTERFERON SUPPRESSES SPECIFICALLY INDUCED ANTIBODY SYNTHESIS *IN VITRO* BY HUMAN LYMPHOCYTES

(Lymphocytes were cultured from a normal human donor immunized 2 weeks previously with Merieux influenza vaccine containing 10 μg A/Bangkok/79 (H3N2) haemagglutinin, 10 μg A/Brazil/78 (HINI) haemagglutinin and 15 μg B/Singapore/79 strain haemagglutinin. All cultures were stimulated with X31 influenza haemagglutinin (5 μg $m^{-1}$), by using the method of Callard (1979), and anti-influenza antibody synthesis was measured after 6 days of culture by the ELISA technique (Callard & Smith 1981).)

| culture conditions (immune lymphocytes in all cultures) | antibody responses (u $ml^{-1}$)† |
|---|---|
| antigen | 12.0±2.0 |
| antigen + interferon‡ | 3.0 |
| antigen + B-cell factor§ | 34.5±2.5 |
| antigen + B-cell factor + interferon | 36.0±4.0 |
| antigen + allogeneic lymphocytes¶ | 56.0±5.5 |
| allogenic lymphocytes + interferon | 48.0±8.5 |

† Mean±s.e.m.

‡ 100 i.u. of interferon-α (Wellcome Research Laboratories batch HSIF, 4.8 log ref. U $ml^{-1}$) added at start of culture.

§ B-cell stimulating factor, 10 vol. % (North & Brenner 1983), added at start of culture.

¶ Antigen-stimulated cells co-cultured with $10^6$ blood lymphocytes from an HLA-incompatible donor.

that bypass the need for inducer T cells, and this form of antibody production is not affected by interferon-α. This observation suggests that T-cell dependent immune responses to novel antigens may be suppressed by virus-induced interferons, whereas established patterns of antibody response may prove resistant. For example, the spontaneous production of polyclonal Ig *in vitro* displayed by circulating B lymphocytes from patients with systemic lupus erythematosus is unaffected by high concentrations of interferon-α (B. K. Pelton, in preparation).

## 10. CONCLUSIONS

Non-specific immunosuppression commonly accompanies virus infections. However, there are relatively few examples of viruses whose virulence depends upon specific suppression of immune responses to the invading virus. There are several mechanisms by which viruses induced immunosuppression. In man, virus growth in selected lymphocyte subpopulations appears to be the most important. The extent to which infection interferes with specific antiviral immunity has not been clarified. Several viruses persist in human lymphocytes for prolonged periods; the pathological consequences of this persistence deserve further study.

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