RECENT ADVANCES IN 3 CLINICAL VIROLOGY

Edited by A.P. Waterson

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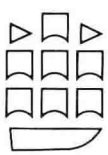

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Recent Advances in
CLINICAL VIROLOGY

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Preface

It is unlikely that any two virologists, faced with the task of choosing subjects for a volume such as this, would pick the same topics. Indeed, the topics, to some degree, pick themselves. For example, one virus which could scarcely fail to have appeared is cytomegalovirus, and this figures large in Chapters 1–3, whether the patient is newly born, pregnant or immunocompromised. Another, perhaps narrower, field in which there is continued activity with much prevailing uncertainty is the role of Coxsackie viruses in progressive, as opposed to acute, cardiac disease, and this is discussed in Chapter 5. Two reviews are concerned with what may be termed, in a somewhat insular way, exotic infections, i.e. imported arbovirus infections (Ch. 11) and various aspects of rabies (Ch. 12). Two chapters deal with specifically technical subjects, one on the ELISA technique (Ch. 4) and one on the storage and retrieval of clinical virological data (Ch. 13). The remaining chapters are concerned, broadly, with the prevention of viral diseases in man, whether actively by vaccines (Chs. 8 & 9), passively by immunoglobulins (Ch. 6) or by a combination of both of these, with other measures, in hepatitis B (Ch. 10). One contribution (Ch. 7) deals with the molecular background to antiviral chemotherapy.

Like its predecessors this number of *Recent Advances in Clinical Virology* makes no claim to be comprehensive, and no one should be disappointed if his favourite theme does not appear. Nevertheless, it is hoped that these reviews will be of practical value to those involved in the application of laboratory techniques to the control of viral disease in man.

Hammersmith, 1983 A.P.W.

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Contents

1. Virus infections in the immunocompromised

J. O'H. Tobin

The immunocompromised or the immunodeficient individual of all ages is more susceptible to clinical disease when infected by viruses which in normal people may often cause only mild or unrecognised illness. Obvious immunodeficiency can be induced by prematurity, inborn defects, infections, neoplasia, malnutrition or by medication. Normal states such as the newborn period, pregnancy and old age may also alter the immunological state of the individual (Oleske & Minnefor, 1980).

Both T cell and B cell abnormalities or deficiencies may be responsible for both congenital and acquired immunodeficiencies. Some viruses are more likely to cause serious disease if the cell-mediated immunity (CMI) is affected while a poor antibody-mediated immunity (AMI) may encourage others. Herpesviruses, vaccinia, measles and wart viruses are commonly found in CMI deficient states while picornavirus, hepatitis B, herpes simplex and influenza viruses are more influenced by poor antibody mediated immunity. Often it is difficult to decide which system is most at fault as many viruses will avail themselves of a defect in either or both systems (Tables 1.1–1.3). Defective phagocytic cells may also be involved as in diabetes or during cytotoxic therapy. In some diseases, sickle cell anaemia and cystic fibrosis specific defects have not been determined (Young, 1981).

Table 1.1 Primary immunodeficient states

1. *T lymphocyte deficiency states*
   Cell-mediated immunity (CMI)
   a. Severe combined immunodeficiency
   d. Thymic hyperplasia (diGeorge's syndrome)
   c. Wiskott-Aldrich syndrome
   d. Nezelot's syndrome.

2. *B lymphocyte deficiency states*
   Antibody mediated immunity (AMI)
   a. Agammaglobulinaemia
   b. IgA deficiency
   c. Defective maturation of B cell function

Anatomical and physiological disturbances (Table 1.4) will affect local sensitivity to viral infection. Herpes simplex virus and vaccinia infection of eczematous skin (Hanshaw & Dudgeon, 1978) and enhancement of symptoms by respiratory viruses in asthmatics (Minor et al, 1974) are examples. Even stress states have been associated with the activation of herpes simplex virus, herpes zoster or human wart virus (Oleske & Minnefor, 1980).

Much of our information of viral effects in the immunocompromised is derived

Table 1.2 Secondary immunodeficiencies (CMI and/or AMI)

1. *CMI predominant defects*	
a. Medication	
(i) cytotoxic drugs	(iii) Radiation
(ii) corticosteroids	(iv) Anti-lymphocytic serum
b. Organ transplantation	
c. Lymphoproliferative Disease	
(i) Hodgkin's Disease	(iii) Acute lymphocytic leukemia
(ii) Lymphoma	
d. Others	
(i) Sarcoidosis	(ii) Leprosy
e. Malnutrition	
f. Natural states	
(i) Pregnancy	(ii) Advanced age
2. *AMI predominant defects*	
(i) Chronic lymphocytic leukemia	(iii) Secondary agammaglobulinaemias
(ii) Multiple myeloma	

Table 1.3 Predominant deficiency not known

a. Genetic disorders
(i) Down's syndrome
(ii) Gaucher's disease
(iii) Duncan's syndrome
b. Collagen disease
c. Uremia/hemodialysis
d. Late malignant disease
e. Newborn and premature

Table 1.4 Anatomical and physiological defects

a. Skin
(i) Eczema
(ii) Burns
b. Splenectomy
c. Asthma
d. Smoking
e. Stress states

from patients treated for malignancy or undergoing renal, cardiac or marrow transplantations.

Members of the Herpesviridae are those most often diagnosed as causing serious disease in the individual and include cytomegalovirus, varicella zoster, herpes simplex and Epstein-Barr viruses.

CYTOMEGALOVIRUS (CMV)

This virus is probably the most important and frequent cause of serious disease in the immunodeficient. It has a world-wide distribution; in tropical countries infection occurs earlier in life than in temperate zones, where infection is acquired slowly throughout childhood, adolescence and young adulthood (Krech & Tobin, 1981).

Once infected, an individual keeps CMV for life, latent in white blood cells and in the urogenital tract, and probably in other systems. It was originally recognised as a cause of congenital infection but following the introduction of organ transplantation a more widespread spectrum of disease has been recognised (Betts & Hanshaw, 1977). It can also be spread by transfusion of blood and its products (Bayer & Tegtmeier, 1976).

Prematurity

CMV infection at any time during pregnancy may lead to congenital disease. Most of its manifestations are not lethal with 95% of infected pregnancies leading to a child who survives (MacDonald & Tobin, 1979).

If associated with prematurity it may be fatal (Ballard et al, 1979). In such infants the virus may be introduced by maternal infection or by exchange transfusions (Benson et al, 1980). In Oxford two fatal cases of CMV infection in infants followed maternal infection in the second trimester with subsequent premature labour and illness and death in the infants a few weeks later. Although these infants were given transfusions all the blood used in Oxford for such exchanges for transplants and leukemic patients or young children undergoing heart surgery is CMV free and so could not have been implicated in these cases. Symptoms in these infants include fever, jaundice, hepato-splenomegaly, respiratory distress, and thrombocytopenia.

Post-transplantation infections

Initially CMV infection was a serious problem in both renal and marrow transplants but with the slow reduction in the amount of immunosuppressive drugs used it is probably less likely to cause fatal illness now (Morris et al, 1982).

Infection is either primary, being introduced into a non-immune subject with the donor material (Warrell et al, 1980), or rarely by blood transfusion. Reactivation of CMV in a recipient already having acquired the virus is more common as the incidence of CMV non-immunes is often low in patients requiring renal and cardiac transplants; it may be high in children receiving marrow grafts.

Renal transplants

The frequency of primary infection with CMV in renal transplant patients will depend on the number of non-immunes amongst the recipients and if CMV antibody-free donors are selected for them. In Oxford with 35–40% of non-immunes (74/188 patients) over a quarter of CMV infections, were primary ones, 35% of the non-immunes being infected and all showing signs and symptoms of illness. In those given kidneys from positive donors the incidence of primary infection was 69%.

These figures are similar to series from Minnesota (Howard et al, 1977) with 29.8% infection of non-immunes but lower than a report for fewer patients from California (Chatterjee et al, 1978) where of 6 non-immunes who were infected primarily four died subsequently of fungal or bacterial infections.

Petersen et al (1981) reported that 51% of 184 episodes of fever in 175 transplant patients were due to CMV as compared with 14% bacterial infection, 5% to fungal infection and 13% to rejection episodes; 70% of the fevers were due to CMV; 37.8% lasted over three weeks and these mainly occurred within the first four months after transplantation. 23 of 36 patients with overt CMV disease died mostly complicated with secondary infection (20 of 24 patients). Bacterial and fungal infections uncompli-

cated by CMV had a lower mortality. During the study 1977–1980 the yearly incidence of CMV seemed to be getting less but whether this was related to a vaccine trial initiated during that time or variation in treatment was not clear.

CMV reactivation amongst the Oxford patients was 62% and lower than the 89–97% noted by the above workers. Although many patients reactivate CMV without clinical symptoms in some cases the disease may be severe and progressive.

Continuous virus excretion and the presence of retinitis may indicate a poor prognosis and suggest as low an immunosuppressive regime as possible.

Clinical manifestations found in Oxford following renal transplantation are summarised in Table 1.5 (Warrell et al, 1980). The ulceration of the gut following

Table 1.5 % Symptoms and signs in CMV infection in renal transplants

	Primary (N20)*	Secondary (N55)
Fever > 37.5°C	95	16.5
Leucopenia (< 500 × 10^6/L	65	29
Thrombocytopenia (< 100 × 10°/L)	60	14.5
Atypical mononuclear cells	50	5.5
Abnormal liver function tests	45	3.5
Lower respiratory tract disease	35	9
Hepato and/or splenomegaly	25	2
Percardial rub	10	0
Epigastric pain	10	0
Gut ulceration	0	5.5
Retinitis	0	2
Asymptomatic†	0	51
Onset of symptoms (days) after transplantation	21–63	21–50
Median	41	54

* Cases not complicated by other infections.
† Serological evidence of infection only.

re-activation may be of two types, individual ulcers in which CMV inclusions can be seen and virus sometimes isolated or a more diffuse ulceration initially affecting the colon and spreading up the gut. Although CMV has not been demonstrated in the lesions of these patients, the two fatal cases seen in Oxford were associated in time with CMV re-activation. This syndrome has not been seen in primary CMV infection or in other patients not re-activating their own virus. Involvement of the gut is associated with high mortality and sometimes haemorrhage (Dietheim et al, 1976). Other manifestations of CMV infection include polyneuritis, arthritis, pancreatitis and encephalitis.

The relation of CMV infection to rejection episodes has not been decided. Suwansirikul et al, 1977; Descamps et al, 1978; Pass et al, 1978 and Warrell et al, 1980 could find no definite association while Lopez et al (1974); Betts et al (1977) and Light & Burke (1979) did so. It is probable that because CMV and rejection episodes are most frequent in the first three months after transplantation that only very large series of patients will reveal the true relationship.

In transplant patients with primary CMV infection withdrawal or reduction of azathioprin and some reduction of corticosteroid therapy may not only help to control

the infection but usually is not followed during the acute infection by kidney rejection. Active CMV seems capable of preventing this rejection on its own. CMV is thus both immunosuppressive itself and can be reactivated by those immunosuppressed.

Marrow transplantation

Following marrow transplantation interstitial pneumonitis is a frequent and serious complication. It occurs from soon after this procedure to more than 200 days later with a medium time of 46 days to 60 days. The pneumonitis is associated with graft verses host disease (GVHD) and its treatment and can be complicated by CMV infection (Meyes et al, 1975). Neiman et al (1976) found interstitial pneumonia in 30 of 61 marrow transplant patients with 18 deaths. 45% of patients had active CMV infection either primary or secondary; in 13 of the 18 dying of pneumonitis it was associated with this virus. They found that fatal CMV associated pneumonitis occurred from the 2nd to 8th week with a medium of 46 days. Clinical disease tended to precede virus excretion or sero-conversion as tested by complement fixation. Winston et al (1979), reported similar figures with 18 of 40 marrow transplant patients with active CMV infection but time of onset of symptoms was later (60 days as median) perhaps because of the greater use of cytosine arabinoside in their immunosuppressive therapy. This drug has some action on CMV and could have delayed the onset of clinical symptoms. Involvement of the gut by CMV can occur as it does after renal transplantation (Strayer et al, 1978).

The increased mortality of patients with active CMV infection would suggest that the virus is a pathogen not just a passenger, and exacerbates the effect of GDVH and its treatment. Both groups of workers suggest that poor response in CF antibody is associated with severe disease and often death, a finding also noted in renal transplantation.

The British experience of interstitial pneumonia suggests it is less of a problem than in the United States (Powles et al, 1980) but detailed microbiology of cases is not yet available although fatal CMV infection has occurred in the United Kingdom.

Cardiac transplantation

After cardiac transplantation, in which procedure an average of 12.6 units of blood was used per patient, nine of 28 CMV non-immune patients, as compared with two of 21 of those with antibody (Rand et al, 1978), died within 90 days. Ten patients had pneumonia or lung abscess. Six of 12 primary cases had their symptoms within 90 days as compared with one of 20 with antibody before hospitalisation and none of non-immunes who were not infected. Fungal infections did not seem affected by CMV infection. An increase of pulmonary infection by other micro-organisms occurred in cardiac transplant patients undergoing primary CMV infection.

Lymphoproliferative disease

In Hodgkin's disease, lymphoma and, especially, in leukemia the frequency of CMV excretion is increased usually without any clinical symptoms (Betts & Hanshaw, 1977). Sometimes non-specific fever, gastrointestinal ulceration or pneumonia to CMV does occur the later two often being life threatening. Caul et al (1972) showed that changes in CMV antibody levels in leukemic patients were often associated with

blood transfusions which suggested that re-infection was required to keep up antibody levels in these subjects. Many leukemic patients lose their CMV antibody after a few months and this lowered AMI and underlying CMI may encourage asymptomatic virus excretion.

Relation of CMV to other conditions

It has been suggested that CMV infection in the normal host enhances other infections such as *Pneumocystis carinii* or even prepare the way for Kaposi's sarcoma (Editorial, 1967; Hymes et al, 1981). Herpes virus particles have been seen in all cultures of this sarcoma and CMV antigens demonstrated in them (Giraldo et al, 1980). This tumour in male homosexuals is being associated with drug inhalation, CMV and other infections in an immunosuppressive condition recently called 'Gay compromise syndrome' (Brennan & Durack, 1981).

In some states such as badly burnt individuals CMV is introduced by blood transfusion (Linneman & McMillan, 1981) and much primary infection of the immunosuppressed is iatrogenic and not acquired from associates.

VARICELLA-ZOSTER VIRUS (VZV) INFECTIONS

VZV causes chickenpox as its primary manifestation and zoster when reactivated (Christie, 1981). It can cause serious disease in those immunodeficient but the majority of cases follow a relatively benign clinical course.

Prematurity and the newborn

Although very rarely causing congenital infection, in the newborns of mothers developing chickenpox within 5–6 days before delivery the mortality may be 10–20% unless VZV immunoglobulin is given promptly after delivery (Meyers, 1974).

Transplantation

Primary chickenpox following renal and cardiac transplantation is rare as most patients are immune; in these patients a history of chickenpox may be a better indication of immunity than laboratory tests unless a sensitive method is employed (Cradock-Watson et al, 1981). Herpes zoster is more common but only a third of reactivations of VZ, as indicated by significant antibody rises, are accompanied by clinical signs. These are usually mild but serious disease has been reported on some occasions. After renal or marrow transplantation the disease does not seem to be especially common in the following three months. It can occur at any time but usually after reactivation of HSV and CMV (Warrell et al, 1980; Atkinson et al, 1980). In Oxford after renal transplantation it was 10 times the expected rate in normal adults.

Lymphoproliferative disease

Severe chickenpox and zoster have been reported in patients undergoing corticosteroid and other therapy for these conditions with an increased fatality especially in those with leukemia (Feldman et al, 1975; Bishop et al, 1981). The relationship between therapy and the underlying disorder is not clear. Although steroids have been

used successfully in treatment of severe haemorrhagic chickenpox long-term therapy with these drugs is associated with an increased risk to VZV especially in leukemia. The identification of these at special risk is difficult and has not been studied extensively to see if they could be identified. Both CMI and AMI are affected in patients with lymphoma (Arvin et al, 1980).

Other conditions

Herpes zoster occurs at all ages but is serious only in those normal people over fifty and this is attributed to age-associated reduction in their immune status. In this age group there are more individuals undergoing therapy for malignancy or other conditions and many of those seen in hospital are under treatment with cytotoxic or other immunosuppressive drugs. In these elderly patients zoster and its associated neuralgia can be severe.

HERPES SIMPLEX VIRUS (HSV)

The commonest manifestation of this infection is cold sores and these have historically always been associated especially with pneumonia and malaria both of which reduce immunity. However, activation of HSV giving cold sores may follow many other stimuli.

Prematurity and newborn

Neonatal HSV infection is a rare disease (Hanshaw & Dudgeon, 1978) associated in the USA mainly with maternal genital infection with HSV type 2; in the United Kingdom the proportion of HSV type 1 seems higher but the number of cases studied is small (Tobin, J O'H, 1975). Mortality in these infants is high in both the generalized infection and encephalitis, but in other neonatal infections without serious complications the disease is often mild or even not recognised (Young & Tobin, unpublished results). Infection of premature infants is likely to be more severe than in full-term infants.

Transplantations

Cold cores are very common after transplantation mainly occuring in the first month following this procedure. Subsequently the infections become less common returning often to the same frequency as before transplantation. In a few cases the lesions may spread to involve the face and eye and very rarely spread down the alimentary tract giving a severe gastroenterocolitis which is usually fatal. After transplantation nearly all clinical manifestations of HSV are reactivations (Rinaldo et al, 1976; Warrell et al, 1980).

Other diseases

In the normal individuals generalised HSV infection is rare but a few fatal cases have been described all in those suffering from some sort of malignancy (Flewett et al, 1969). In Kaposi's varicelliform eruption the virus may attack the damaged eczematous skin and produce a severe disease especially in infants (Hanshaw & Dudgeon, 1978).

EPSTEIN-BARR VIRUS (EBV)

The fourth herpesvirus EBV was until recently not considered too important in the immunosuppressed as it seemed to cause little obvious disease. It has been associated with GVHD after marrow transplantation (Sullivan et al, 1978). The relation of EBV (Epstein & Achong, 1977) to Burkitt's lymphoma (de Thé et al, 1978) and nasopharangeal carcinoma has been extended to malignancies in immuno-deficient individuals (Portillo, 1980). The incidence of lymphomas following transplantation is increased especially so if cyclosporin A is used as an immunosuppressive (Calne et al, 1979). Oropharangeal excretion of EBV by patients with lymphoproliferative disorders or following renal homografts is considerably raised above that found in the normal population (Strauch et al, 1974; Shimon Chang et al, 1978). Nagington & Gray (1980) suggested a relationship between EBV and lymphomas in transplant recipients and soon afterwards Crawford et al (1980) demonstrated EBV antigen in a lymphoma from such a patient. Hanto et al (1981) reported two cases of lymphoma associated with EB virus five and eight years after transplantation, the tumour originating in the palate and tongue in contrast to Crawford's case in which the first sign was an inguinal swelling. In one of Hanto's cases the tumour resolved but the other was fatal with proliferation in the liver, bone marrow and other organs. EBV serology and demonstration of its genome in the tumours indicated the role of EBV as causal. The proliferation of lymphoid tissue following EB virus infection is polyclonal but tumours are monoclonal indicating that immunological surveillance need only allow a single changed cell to escape detection for a cancer to occur. It is thought that the fundamental defect is suppression of memory T cells responsible for the repression of infected B cells (Crawford et al, 1981).

DIAGNOSIS OF HERPESVIRUS INFECTIONS

In the absence of classical and clinical signs, i.e. zoster or chickenpox, or when modified by immunosuppression, laboratory tests are essential for exact diagnosis. These infections can be diagnosed by: (1) Identification of the agent in specimens by immunological or other means; (2) by isolation of the infecting agent; and (3) by serology on serum samples.

1. HSV and VZ can be identified in skin lesions by electron microscopy or immunofluorescence although the former needs confirmation subsequently by culture (Juel Jensen & MacCallum, 1972). It is sometimes possible to see CMV in urine deposits either as the characteristic owl's eye inclusion under the light microscope or the virus particles by electron microscopy. This is not as sensitive as cultural methods but if facilities for cytology (Traystman et al, 1980) and electronmicroscopy are readily available can be used on urine and respiratory tract specimens; as an early method of diagnosis the chances are not high for a positive result. Immunofluorescence has occasionally indicated the virus in sputum but the failure rate compared with culture is high. At autopsy CMV inclusions can readily be recognised in histological sections by normal staining methods. Immunofluorescence using a human CMV positive serum is not very satisfactory and specific animal sera are different to make.
2. HSV, VZ and CMV can be isolated in human embryo lung or foreskin fibroblast