

Diagnostic assays in cytomegalovirus retinitis

A variety of viruses have been associated with retinal and choroidal disease, including the mumps virus, rubella virus, measles virus, coxsackievirus B4, arbovirus, human immunodeficiency virus, and members of the human herpesvirus family. This latter group causes a wide range of clinical syndromes with varying degrees of severity. Of particular concern to the ophthalmologist is the potentially devastating effect such infections may have on vision when the retinal and choroidal tissues are involved. At present, diagnosis of these conditions relies heavily on recognition of the clinical signs at funduscopy which can be difficult or, at times, misleading. Recently, there has been considerable interest in developing and assessing laboratory based assays to supplement clinical examination. Doornenbal *et al* address this problem in this issue of the *BJO* (p 235).

Herpesviruses that have been implicated as causes of, or associated with, retinochoroiditis are herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpesvirus 6 (HHV-6). These viruses have been associated with CMV retinopathy, acute retinal necrosis (ARN), progressive outer retinal necrosis, and multifocal choroiditis. HHV-6 has been found in association with CMV and HIV in retinal tissue of patients with AIDS but has not been specifically associated with a clinical condition.¹

Human CMV infects most of the population at some time in their life. Over many generations genetically different strains of CMV have emerged which continually circulate throughout the world.² Primary CMV infection is often followed by persistent or recurrent infections which may be caused either by reactivation of latent virus or by reinfection by an antigenic variant. It is rare for CMV infection of the healthy adult to lead to ocular infection, although there have been isolated reports.³ In contrast, ocular infection is common in congenital CMV infection and in patients who are immunocompromised, such as those with AIDS, and CMV retinitis has also been transmitted via blood transfusion with CMV antibody titres greater than 1:8.^{4 5}

As stated above, the diagnosis of CMV retinitis relies on the fundus appearance. However, in the early stages of disease and in patients with atypical features, it is difficult to differentiate between retinitis caused by CMV and retinitis associated with the other herpesviruses. Discrimination between viral and non-viral pathogens such as *Toxoplasma gondii* can be particularly difficult by clinical examination alone. Rapid accurate diagnosis of ocular CMV infection and the prompt initiation of appropriate therapy is essential both for the preservation of sight and the improved survival of the patient. Furthermore, the personal cost to the patient and the waste of resources associated with the use of multiple antibiotic and antiviral therapies prompts the development of rapid, sensitive, and specific diagnostic tests for ocular pathogens such as CMV.

In this issue of the *BJO* Doornenbal *et al* assess two possible diagnostic strategies for the detection of ocular herpesvirus infection. The first, based on the polymerase chain reaction (PCR), is an example of a method which relies on the direct detection of virus or part of the viral genome, and the second, based on demonstration of a specific immune response, is an example of an indirect method relying on the host reaction to the presence of virus. Both methods are not without their difficulties, in

part because of the specific circumstances surrounding ocular infections and the immunocompromised host.

Appropriate biopsy site selection is essential with direct detection methods and ideally in these infections this would be the retina, but for obvious reasons retinal tissue is not usually available. Although the isolation of CMV from the blood and peripheral sites confirms that a patient has active viral replication,⁶ these findings are not diagnostic for target organ disease. Indeed, the majority of AIDS patients may have culturable CMV viraemia without evidence of ocular disease. Viral culture of ocular fluids has not been useful in the past because of the minute sample volume which is available and the relative insensitivity of *in vitro* propagation, perhaps as a result of neutralising antibodies. The technique of PCR^{7 8} allows the amplification of nucleic acid sequences to easily detectable levels, making the direct detection of viral genome in small ocular fluid samples a possibility for the first time. In Doornenbal *et al*'s study, aqueous, vitreous, and subretinal fluid samples were taken at various times in the disease process, which for clinical reasons may have been unavoidable but such samples are not directly comparable.⁹

The conclusion in Doornenbal and colleagues' paper that PCR based assays applied to ocular fluids are specific and sensitive tools in the diagnosis of CMV retinitis in patients where the clinical diagnosis is unclear, confirms findings in two recent studies using standardised PCR based assays.^{10 11} As Doornenbal and colleagues state, it is now time to develop generally standardised protocols and intercentre quality control programmes to allow both better comparison of results from different laboratories and the sharing of data in the case of the rarer ocular infections.

The unique contribution of this paper is to compare these PCR based assays with antibody based assays in the form of the Goldmann-Witmer quotient (GWq). This method determines the ratio of antibody titres against suspected intraocular pathogens in ocular fluids and serum to the total amount of IgG, and has been applied successfully in the past to patients with a diagnosis of ocular toxoplasmosis and ARN in the immunocompetent patient.^{12 13} As such, this was an appropriate choice of assay for comparison with clinical diagnosis and PCR based assays, but as the authors themselves point out there was little expectation of it proving to be useful in patients with AIDS, as for a variety of reasons they have notoriously variable IgG responses.¹⁴ Their results confirmed this suspicion.

This paper raises many interesting points relating to the significance of detection of herpesviral genome in ocular fluids in patients with a variety of forms of uveitis, as well as confirming that sensitive and specific PCR based assays for CMV retinitis now exist. Further understanding of herpesviruses affecting the eye should promote better therapeutic intervention in many ocular inflammatory disorders in the future.

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