BK Virus Specific IgM Responses in Cord Sera, Young Children and Healthy Adults Detected by RIA

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With 3 Figures
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Summary
An IgM capture solid-phase radioimmunoassay (MACRIA) for BK virus (BKV) specific IgM is described. This test was found to be more sensitive in detecting BKV specific IgM than both haemagglutination inhibition and immune electron microscopy with serum fractions from sucrose density gradients. The use of this specific assay allowed large numbers of sera to be examined with ease so that the distribution of BKV specific IgM in different populations could be studied more fully.

BKV specific IgM was detected in 11/300 sera from London blood donors, in 24/114 sera from children aged between 2 and 11 years admitted to a paediatric unit and 14/79 sera taken from children aged between 2 and 5 years for the investigation of anti-streptolysin 0 titres. BKV specific IgM was not detected in 404 cord sera examined to investigate the transplacental transmission of BK virus.

Introduction
The human polyomaviruses BK (BKV) and JC (JCV) were first isolated in 1971 (8, 17). Following these observations sero-epidemiological studies have shown that primary infection with BKV occurs predominantly in childhood, with the peak incidence of infection occurring between the ages of 2 and 5 years. In contrast infection with JCV occurs later with antibody detected only rarely in children aged between 2 and 10 years (5). Both viruses are thought to persist in man and reactivation has been demonstrated in different groups of immunocompromised patients (2, 19, 24). Reactivation of both human polyomaviruses has also been demonstrated in pregnant women (4).
Primary infection with BKV in healthy children has not been associated with any specific clinical syndrome, but BKV has been linked with the development of ureteric obstruction following renal transplantation (3). JCV is the cause of progressive multifocal leukoencephalopathy (PML), an uncommon neurological disease which occurs in immunocompromised patients. It is not clear whether this disease is due to a primary JCV infection or to the reactivation of latent virus.

BKV specific IgM has been demonstrated in healthy children (21), healthy adults and renal transplant recipients (7) and also in pregnant women (4, 22). Several studies have been performed to examine the transplacental transmission of BKV by searching for BKV specific IgM in cord sera (1, 4, 10, 20, 22, 23). Investigations employing different techniques have given conflicting results.

To study the possible transplacental transmission of BKV and to examine the BKV specific IgM response in different populations we have developed a solid phase IgM antibody capture radioimmunoassay (MACRIA) for BKV specific IgM. This assay enables large numbers of sera to be screened with ease.

**Materials and Methods**

**Standardisation of Reagents**

**Virus**

Prototype BKV was grown in a human embryo lung cell line; three different antigen preparations were made from this. (1) Supernate was harvested after 23 days incubation. (2) The cells and supernate were harvested together after 27 days incubation by 3 cycles of freezing and thawing, followed by sonication and clarification. (3) The cells from a 4 oz bottle were harvested after 27 days incubation into 2 ml of PBS by 3 cycles of freezing and thawing, followed by sonication and clarification. All 3 preparations were stored at $-40^\circ\text{C}$.

**BKV Antiserum**

This rabbit antiserum was a gift from Dr. Giraldo, Memorial Sloan-Kettering Cancer Center.

**¹²⁵I-Labelled Anti-Rabbit Antiserum**

Anti-rabbit Ig, ¹²⁵I-labelled donkey whole antibody was obtained from Amersham International (Code IM.134).

**Assay for the Detection of BKV Specific IgM**

The technique of IgM antibody capture as described by Flehmig et al. (6) was employed for the detection of BKV specific IgM. Etched polystyrene beads diameter 6.5 mm (Northumbria Biologicals) were used as the solid phase. They were coated in a 1:3000 dilution of goat anti-human IgM (Tago Inc.) made up in 0.05 M carbonate/bicarbonate buffer, pH 9.6, by incubating at room temperature for 2 hours and then overnight at 4°C. The beads were stored in this coating buffer for up to 1 week before use.

For the test the anti-µ coated beads were washed three times in PBS Tween buffer (Don Whitley Scientific Ltd.) and transferred to a 20 well reaction tray.