CHAPTER 5

Serological Cross Reactivity between Polyomavirus Capsids

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Abstract

Multiple methods have been used to measure antibodies to polyomavirus virions. In order to have a common method for all polyomaviruses, we developed enzyme immunoassays (EIAs) using virus-like-particles (VLPs) produced in the baculovirus expression system. We tested serum samples from humans and rhesus macaques in VLP-based EIAs for the two human polyomaviruses, BK and JC virus, and two nonhuman primate polyomaviruses, simian virus 40 (SV40) and lymphotropic polyomavirus (LPV). Rhesus sera exhibited low level reactivity to BK and JC, and approximately 10 and 15% of human sera showed low level reactivity to SV40 and LPV, respectively. Competitive inhibition assays with VLP protein demonstrated that the reactivity of rhesus sera against BK and JC VLPs was blocked by both SV40 and the respective human polyomavirus, indicating that the BK and JC assays were detected cross-reacting antibodies Similarly, the reactivity of the majority of human sera to SV40 was blocked by both SV40 and BK or JC, demonstrating that the SV40 reactivity of human sera is largely due to cross reacting BK and JC antibodies. In contrast, the reactivity of human sera to LPV VLPs was blocked by LPV but not by BK or JC, providing serological evidence for an unknown human polyomavirus related to LPV. SV40 and LPV VLP-based EIAs and competitive inhibition assays with heterologous VLPs provide tools for seroepidemiological studies of possible SV40 and LPV-like infections of humans.

Introduction

Polyomaviruses are small nonenveloped DNA viruses. They are widely distributed in nature and have been described from humans, monkeys, cows, rabbits, mice, hamsters, chickens and parrots. The presently known human polyomaviruses are BK virus (BKV) and JC virus (JCV). Most primary infections with BKV and JCV occur in childhood and are asymptomatic. The viruses persist indefinitely as latent infections in the kidneys and B lymphocytes and can reactivate in times of immunological impairment. Reactivation of JCV may result in progressive multifocal leukoencephalopathy, a subacute demyelinating disease of the central nervous system.[1] BKV reactivation principally manifests in the kidneys as hemorrhagic cystitis or nephropathy.[2,3] Simian virus 40 (SV40) is a natural infection of some species of Asiatic macaques including the rhesus macaques of north India. Human exposure to SV40 occurred on a wide scale between 1955 and 1963 due to contamination of some lots of inactivated poliovaccines.[4] The recent detection of SV40 genomic sequences in human tumors has raised a question of SV40 infection in humans.[5-9] There is indirect evidence for human infection with another polyomavirus based on reactivity of human sera with a lymphotropic polyomavirus (LPV)of African green monkeys.[10,11]

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Hemagglutination inhibition (HI) assays have been the standard method for measurement of antibodies to BKV and JCV, and a virus infectivity neutralization assay has been the standard method for detection of SV40 antibodies.[12-16] There is no widely accepted standardized method for measurement of antibodies to LPV. Enzyme immunoassay (EIA) technology has become the preferred method for measurement of antiviral antibodies because EIA provides greater sensitivity and precision compared with HI and neutralization assays. EIA is also more economical for large-scale seroepidemiological studies. We recently established EIAs for BKV, JCV, SV40 and LPV using virus-like particles (VLPs) as antigens.[17] The capsid proteins of a wide range of viruses, when expressed in insect cells, yeast or *E. coli*, self assemble into empty particles that resemble native virions morphologically and antigenically. Polyomavirus capsids are composed of 72 capsomeres arranged on a T = 7 icosahedral lattice. The capsomers are pentamers of the VP 1 major capsid protein, which forms the outer shell of the capsid. In addition to the 360 molecules of VP1, the native virion contains approximately 1 to 10 molecules of the minor capsid proteins VP2 and VP3. The VP1 proteins of several polyomaviruses have been expressed in yeast or insect cells and shown to self assemble into a capsid-like structure in the absence of the VP2 and VP3 proteins.[18-26] A 3D reconstruction of BKV-VLPs revealed a structure similar to that of native polyomaviruses.[26] VLPs have proven to be exceptionally good reagents for EIAs because they display surface conformational epitopes, which are often the immunodominant, type or species-specific and neutralizing epitopes. The availability of VLP-based EIAs for multiple polyomaviruses has made it possible to perform comparable serological assays for all the viruses and to evaluate cross-reactivity by adsorption studies. In this chapter we describe our detailed studies of serological cross reactivity between BKV, JCV, SV40 and LPV capsids using VLP-based EIAs.

Virus-Like Particle-Based Polyomavirus Enzyme Immunoassays

We obtained recombinant baculoviruses expressing the VP1 major capsid protein of BKV and JCV from Stephen Frye and Peter Jensen and constructed recombinant baculoviruses expressing the VP1 coding sequences of SV40, LPV and mouse polyomavirus (MPV) using the Bac-to-Bac baculovirus expression system. We purified VLPs from lysates of insect cells infected with the recombinant baculoviruses by CsCl density gradient ultracentrifugation and cation exchange or gel exclusion liquid chromatography.[17,27] SDS-PAGE analysis of purified VLPs of SV40, BKV and JCV showed a major protein band of about 40, 43 and 40 kDa, respectively, and electron microscopy of the VLP containing preparations revealed particles with a diameter of 45-50 nm, morphologically consistent with empty polyomavirus capsids (Fig. 1). The VP1 protein of LPV was approximately 40 kDa and formed 50 nm particles (data not shown). The optimum concentration of polyomavirus VLPs and serum dilution used in the EIA was determined by titration of positive and negative controls. As the assays are currently configured, wells of PolySorp microtiter plates (Nunc, Naperville, IL) are coated overnight at 4°C with 20 to 30 nanograms of total protein per well and serum samples are tested at a 1:400 dilution. The plates are prepared the day before use and then blocked for 2 hours at room temperature with 0.5% (wt/vol) polyvinyl alcohol, MW 30,000-70,000 (Sigma, St Louis MO) in PBS (0.5% PVA). Serum samples are left to react for 1 h at 37°C and antigen-bound immunoglobulin is detected with peroxidase-conjugated goat antibodies against human IgG (Zymed, San Francisco, CA), diluted 1:2000 in 0.5% PVA, 0.025% Tween 20, 0.8% (wt/vol) polyvinylpyrrolidone, MW 360,000 (Sigma) in PBS. After 30 min at 37°C, color development is initiated by the addition of 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) hydrogen peroxide solution (Kirkegaard and Perry, Gaithersburg, MD). The reaction is stopped after 20 min by addition of 1% dodecyl sulfate and absorbance is measured at 405 nm, with a reference wavelength of 490 nm, in an automated microtiter plate reader (Molecular Devices, Menlo Park, CA).

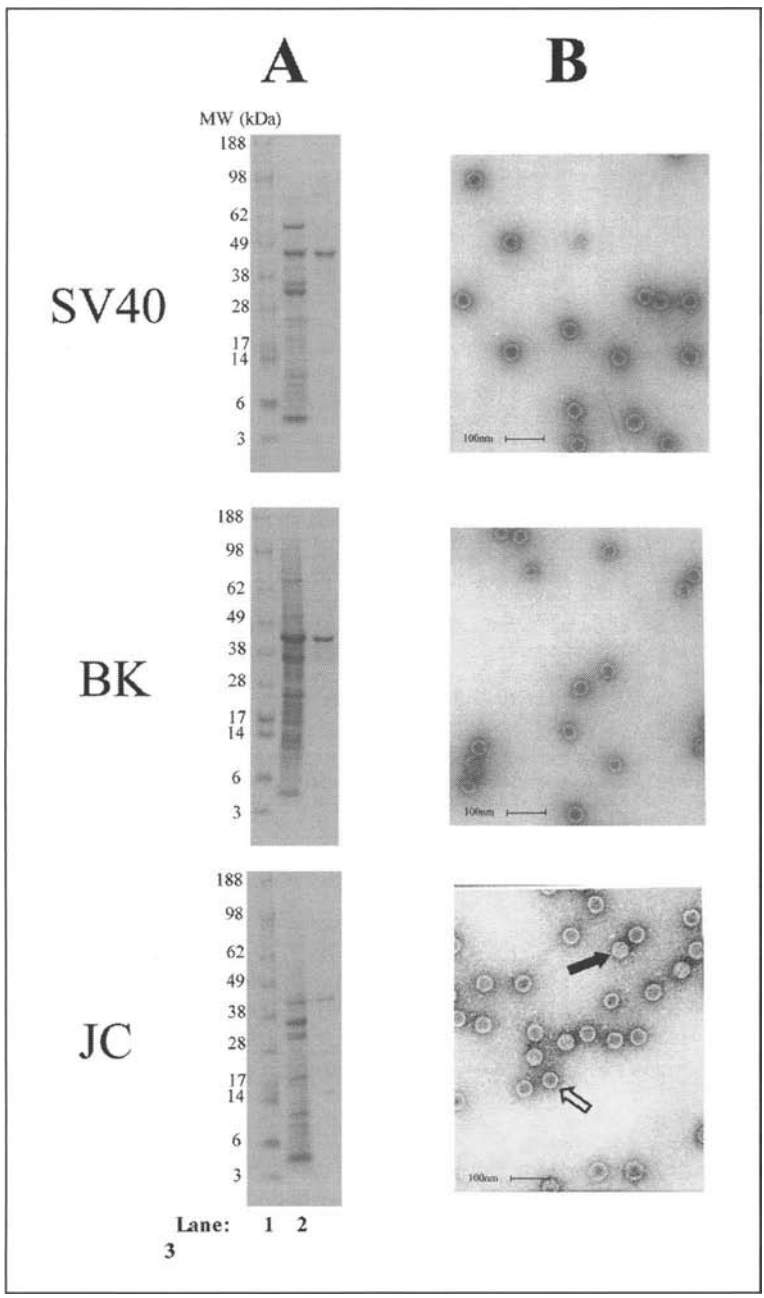

Figure 1. SDS-PAGE of lysates of insect cells infected with recombinant baculoviruses expressing SV40, BKV or JCV VP1 protein and of purified SV40, BKV and JCV VLPs (A) and transmission electron micrographs (x 105,000 magnification) of SV40, BKV, and JCV VLPs (B). In the electron micrograph, the open arrow points to an empty particle and the solid arrow to a full particle. From: Clin Diagn Lab Immunol 2003; 10(2):278-85, ©2003 American Society for Microbiology, with permission.

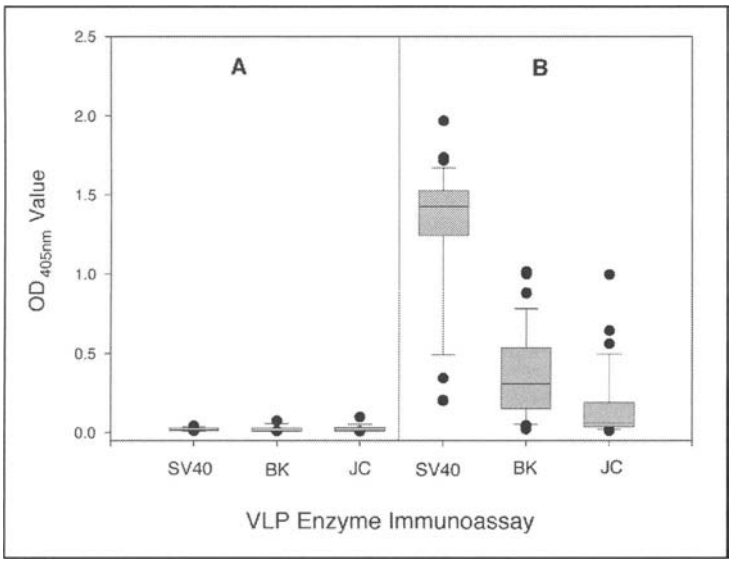

Figure 2. Reactivity of 17 SV40 neutralizing antibody negative (A) and 39 antibody positive (B) rhesus macaque sera in SV40, BKV and JCV VLP-based enzyme immunoassays. The length of the box corresponds to the 25-75% interquartile range. The horizontal line in the box indicates the median optical density value. The lines extending upward and downward from the box mark the 10th to 90th percentile range. Outlier values are shown as closed circles. From: Clin Diagn Lab Immunol 2003; 10(2):278-85, ©2003 American Society for Microbiology, with permission.

Reactivity of Rhesus Macaque Sera in VLP-Based Polyomavirus Enzyme Immunoassays

We tested rhesus sera known to be SV40 antibody–negative and antibody-positive by virus plaque neutralization tests for reactivity to SV40, BKV and JCV in the VLP EIAs (Fig. 2). The SV40 VLP EIA had 100% sensitivity and 100% specificity when compared with the reference standard of SV40 neutralization assay. The SV40-negative rhesus sera were nonreactive with all three VLPs, but the SV40 antibody-positive sera were clearly reactive with both BKV and JCV VLPs, but to a much lesser extent than with SV40 VLPS. It is unlikely that the reactivity is due to exposure of monkeys to BKV or JCV from human handlers because the BKV and JCV seroreactivity was not observed in SV40 antibody-negative sera, despite the fact that these macaques had the same risk of exposure to BKV and JCV. The most likely explanation for BKV and JCV reactivity of macaque sera is cross reacting antibodies elicited by SV40 infection. To further document cross reactivity, we developed competitive inhibition assays. Inhibition of seroreactivity to a particular VLP only with homologous VLP would be evidence of specific antibodies, whereas inhibition of seroreactivity to a particular VLP by a heterologous VLP would be evidence of cross reactivity. In preliminary experiments the reactivity of an SV40 antibody positive rhesus serum in the SV40 VLP-EIA was shown to decrease exponentially in the presence of increasing concentrations of SV40 VLPs in the diluent for the serum (Fig. 3). A concentration of 4 ug of SV40 VLP protein per ml resulted in near maximal inhibition of SV40 seroreactivity. Similar curves were obtained with other SV40 antibody positive rhesus sera (data not shown). We tested 34 SV40 antibody positive rhesus sera in the SV40 VLP EIA in the presence of 4 ug/ml of competing SV40, BKV, JCV, LPV or MPV VLP protein. The percent inhibition of SV40 reactivity by each VLP was calculated as $1 - OD_{competing\ VLP}/OD_{buffer\ control} \times 100$. In Figure 4, the percent inhibition by SV40 VLP protein is plotted versus percent inhibition by BKV VLP protein. SV40 seroreactivity was inhibited from 1.5%

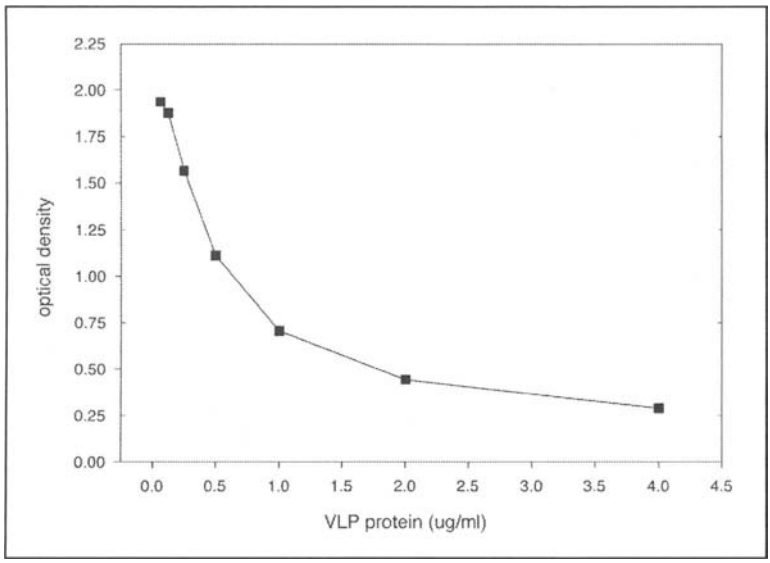

Figure 3. Competitive inhibition of SV40 reactivity of an SV40 antibody positive macaque serum by increasing concentrations of SV40 VLP protein. The serum sample was tested in the SV40-VLP EIA in the presence of serial 2-fold dilutions of SV40 VLP protein starting at a concentration of 4ug/ml. The assay was completed by the sequential addition of peroxidase-labeled goat anti-human IgG and ABTS substrate. The plate was read at 405 nm in an automated microtiter plate reader.

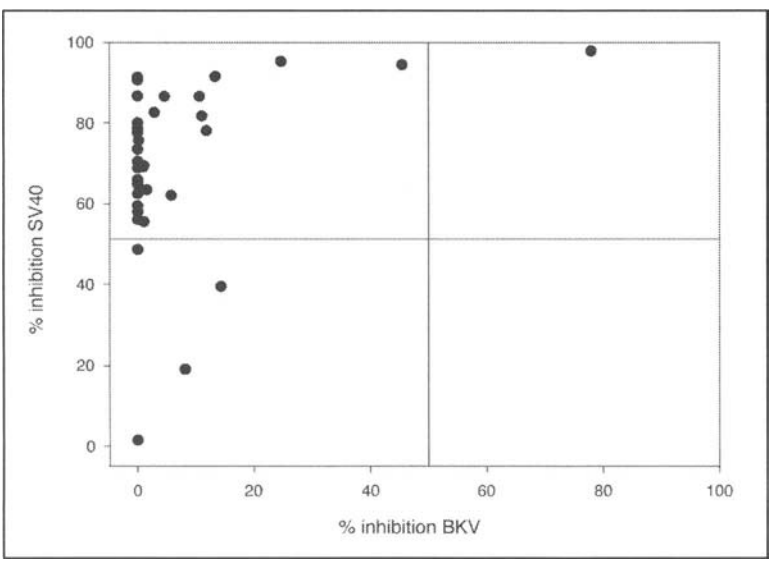

Figure 4. Scatter plot of percent competitive inhibition of SV40 reactivity of 34 macaque sera by SV40 and BKV VLP protein. Serum samples were tested in the SV40-VLP EIA in the presence of 4ug/ml of SV40 or BKV VLP protein. The assay was performed as described in the legend to Figure 3. Percent inhibition by each VLP was calculated as $1 - OD_{competing\ VLP}/OD_{buffer\ control} \times 100$. The percent inhibition by SV40 VLP protein (y-axis) is plotted versus percent inhibition by BKV VLP protein (x-axis). The horizontal and vertical lines mark 50% inhibition by SV40 and BKV VLP protein, respectively.

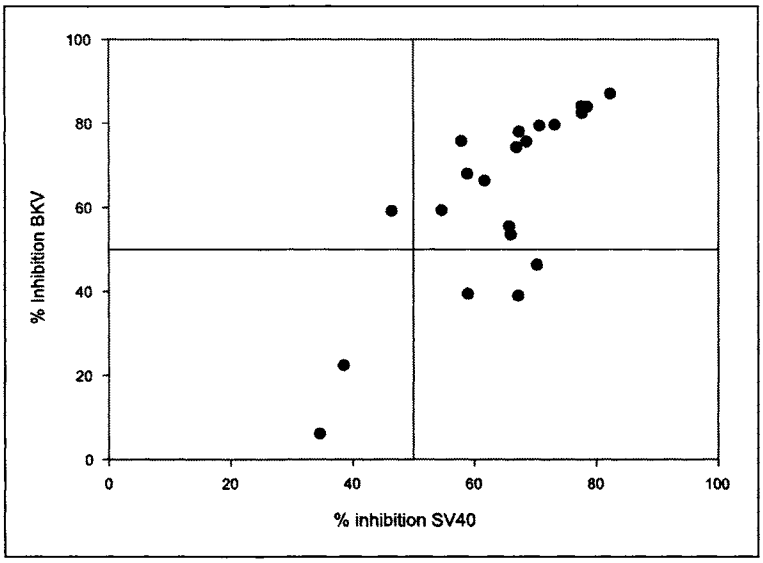

Figure 5. Scatter plot of percent competitive inhibition of BKV reactivity of 21 macaque sera by SV40 and BKV VLP protein. Serum samples were tested in the BKV or VLP EIA in the presence of 4ug/ml of SV40 or BKV VLP protein. The assay was performed as described in the legend to Figure 3 and percent inhibition was calculated as described in the legend to Figure 4. The percent inhibition by SV40 VLP protein (y-axis) is plotted versus percent inhibition by SV40 VLP protein (x-axis). The horizontal and vertical lines mark 50% inhibition by BKV and SV40 VLP protein, respectively.

to 98% by SV40 VLPs and from 0% to 78% by BKV VLPs. If horizontal and vertical reference lines are drawn at the 50% inhibition level for SV40 and BKV VLPs, respectively, the graph divides into four quadrants. We defined reactivity as SV40 specific if sera fell in the upper left quadrant (>50% inhibition by SV40 VLPs and <50% inhibition by BKV VLPs), as nonspecific if sera fell in the lower left or right quadrants (<50% inhibition by the SV40 VLPs), and cross reactive if sera fell in the upper right quadrant (>50% inhibition by SV40 and BKV VLPs). Based on these cut points, the reactivity of 29 (85%) of 34 SV40 antibody positive macaque sera was SV40 specific; the reactivity of 4 (12%) sera was nonspecific; and that of 1 (2.9%) serum sample was due to BKV cross reacting antibodies. In competitive inhibition assays with the other heterologous VLPS, we found that SV40 seroreactivity of macaque sera was never inhibited by more than 50% percent by JCV, LPV or MPV VLPs (data not shown). Thus, the data support the specificity of SV40 VLP reactivity of rhesus sera for SV40. When 21 rhesus sera reactive in the BKV VLP EIA were tested in competitive inhibition assays (Fig. 5), 15 sera were scored as cross reactive, 5 as nonspecific and one as BKV specific. The few sera that reacted to JCV VLPs were inhibited by both SV40 and JCV VLPs (data not shown). Thus, the antibodies in rhesus sera that react with BKV and JCV most likely are induced by SV40 infection and cross react with the human polyomaviruses.

Reactivity of Human Sera in VLP-Based Polyomavirus Enzyme Immunoassays

The detection of BKV and JCV cross reactive antibodies in sera of rhesus macaques infected with SV40 prompted us to test for the reciprocal response in human sera. We looked for evidence of cross reacting antibodies to SV40 in individuals infected with BKV or JCV. Infections with both BKV and JCV are common and occur early in life. The antibody prevalence to

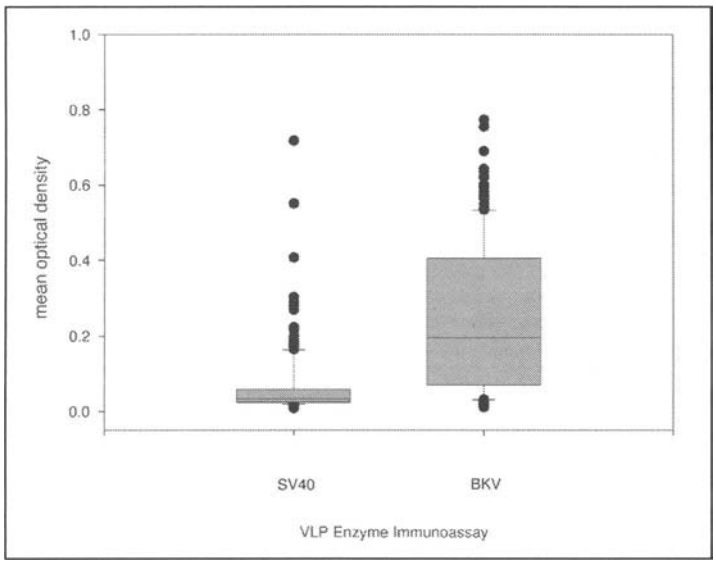

Figure 6. Comparison of reactivity of 586 human sera in BKV and SV40 VLP-based enzyme immunoassays. The control serum samples from a case-control study of lymphoma were tested for reactivity to SV40 and BKV. The length of the box corresponds to the 25-75% interquartile range. The horizontal line in the box indicates the median optical density value. The lines extending upward and downward from the box mark the 10th to 90th percentile range. Outlier values are shown as closed circles.

BKV reaches nearly 100% by the age of 10-11 years and then declines to around 70-80% in the older age groups. The antibody prevalence to JCV reaches a peak of about 75% by adult age.[13,28,29] Because of the controversy concerning human infection with SV40, a number of studies have examined the seroprevalence of SV40 in human populations. The reported prevalence of SV40-reactive antibodies in human sera by neutralization assay is between 3-10%.[16,30-35] The most comprehensive data are reported by Knowles et al[32] who examined over 2400 sera collected in England. The donors ranged in age from 1 to 69 years. The overall SV40 antibody prevalence was 3.2% and did not increase with age of the donor. More recently SV40 seroprevalence in human sera has been measured by VLP EIA. In a case-control study of lymphomas in Spain, we found 9.5% of 587 control sera reactive to SV40 in VLP EIA.[36] Sixty-five percent of the sera contained BKV antibodies. The levels of SV40 antibodies were low as compared to that of BKV antibodies (Fig. 6). In a population-based study of 415 adult sera from Washington State, Carter et al[37] reported 7.7% prevalence by SV40 VLP EIA. The origin of SV40 antibodies in humans is unclear since contact with macaques is rare in most human populations. It is possible that the antibodies reacting with SV40 are induced by the human polyomaviruses and cross-react with SV40 or that they result from infection of the human population with another virus or possibly SV40 from an unknown source.

We tested 67 human SV40 antibody positive sera in competitive inhibition assays to determine the specificity of the response. For 52 (78%) sera, greater than 50% of the SV40 reactivity was competitively inhibited by both SV40 VLP protein and either BKV or JCV VLP protein (Fig. 7). Four (6%) sera were inhibited by less than 50% by SV40 VLPs and were also weakly inhibited by BKV or JCV VLPs, suggesting that the reactivity is either nonspecific or the assay conditions were not optimal. Eleven (16%) sera gave a pattern of reactivity similar to that of SV40 antibody positive macaque sera, with greater than 50% inhibition by SV40 and less than 50% by BKV and JCV VLPs. There was no significant inhibition (>50%) of SV40

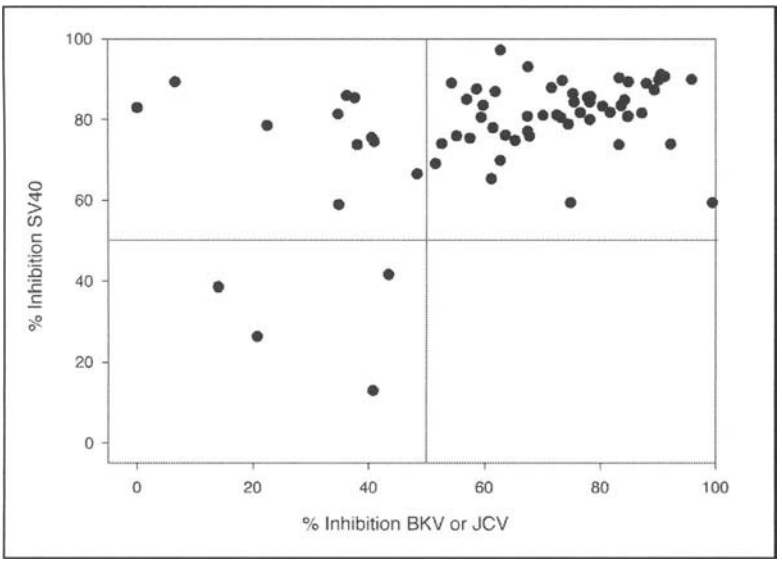

Figure 7. Scatter plot of percent competitive inhibition of SV40 reactivity of 67 human sera by SV40 and BKV or JCV VLP protein. Serum samples were tested in the SV40 VLP EIA in the presence of 4ug/ml of SV40, BKV or JCV VLP protein. The assay was performed as described in the legend to Figure 3 and percent inhibition was calculated as described in the legend to Figure 4. The percent inhibition by SV40 VLP protein (y-axis) is plotted versus maximal percent inhibition by BKV or JCV VLP protein (x-axis). The horizontal and vertical lines mark 50% inhibition by SV40 and BKV or JCV VLP protein, respectively.

seroreactivity by either LPV or MPV VLP protein (data not shown). The profile of reactivity of human sera to BKV and JCV VLPs in the competitive inhibition assays resembled that of macaque sera to SV40 VLPs. BKV seroreactivity was inhibited by BKV VLP protein but not by JCV VLP protein, and conversely, JCV seroreactivity was inhibited by JCV and not BKV VLP protein (Fig. 8). SV40, LPV, and MPV VLP proteins did not significantly inhibit either BKV or JCV seroreactivity (data not shown).

The question whether BKV or JCV antibodies cross react with SV40 has been addressed previously by comparing reactivity to SV40 and human polyomaviruses in serum samples from the same individual. Brown et al[38] examined this question for the first time in their study of 1500 sera from 28 isolated aboriginal populations that had no contact with monkeys and had not received polio vaccines. They found that 5% of 111 BKV-negative sera, and 35% of 40 BKV-positive sera ($p<0.001$) had low levels of neutralizing antibodies to SV40. They concluded that infection with BKV may account for the SV40 reactivity of their sera. In their study, Knowles et al[32] reported that 3.8% and 4.5% of human sera containing, respectively, BKV and JCV antibody, neutralized SV40 as compared to 0.9% and 2.5%, respectively, of BKV-negative and JCV-negative sera. These differences were highly significant. Only one of 79 sera with SV40 antibodies was negative for both BKV and JCV antibodies. Rollison et al[33] found that 11.9% of 96 BKV-positive and none of 20 BKV-negative sera neutralized SV40 ($p=0.08$). Using VLP-based assays, we found a correlation between BKV and SV40 antibioses (Spearman $r = 0.60$, $p<0.001$) and to a lesser extent, between JCV and SV40 antibodies (Spearman $r = 0.18$, $p = 0.06$).[17] In a population based sample of 415 adults sera tested by VLP EIA for all three viruses, Carter et al[37] reported a correlation between BKV and SV40 antibodies (Spearman $r - 0.34$, $p < 0.001$) and also between JCV and SV40 antibodies (Spearman $r = 0.030$, $p < 0.001$). Our competitive inhibition assays provide direct experimental evidence for cross reacting BKV and JCV antibodies as the explanation for the SV40 reactivity of most

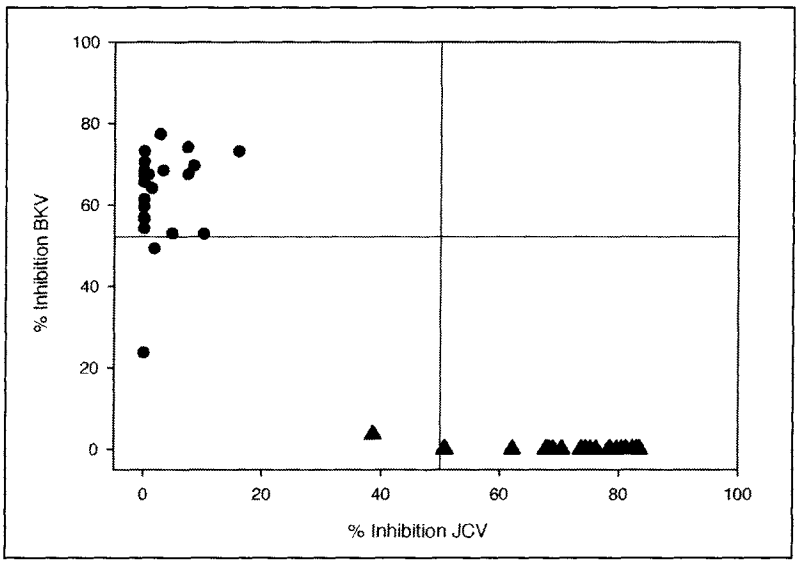

Figure 8. Scatter plot of percent competitive inhibition of BKV and JCV reactivity of 23 human sera by BKV and JCV VLP protein. Serum samples were tested in the BKV (circle) or JCV (triangle) VLP EIA in the presence of 4ug/ml of BKV or JCV VLP protein. The assay was performed as described in the legend to Figure 3 and percent inhibition was calculated as described in the legend to Figure 4. The percent inhibition by BKV VLP protein (y-axis) is plotted versus percent inhibition by JCV VLP protein (x-axis). The horizontal and vertical lines mark 50% inhibition by BKV and JCV VLP protein, respectively.

human sera. Carter et al.[37] also found that preadsorption of SV40-positive sera with BKV or JCV VLPs completely removed their SV40 reactivity.

Reactivity of Human Sera in LPV VLP-Based Enzyme Immunoassay

Lymphotropic polyomavirus was isolated from a B-lymphoblastoid cell line derived from an African green monkey.[39] Analysis of the complete genome sequence of LPV revealed conserved features of the polyomavirus genus and showed that LPV is only distantly related to SV40 and the human polyomaviruses.[40,41] Serological surveys have shown that approximately 30% of humans and all nonhuman primates surveyed, with the exception of baboons, have antibodies to LPV by either immunofluorescence, immunoprecipitation or neutralization assays.[10,11,42] We have detected LPV antibodies by VLP EIA in approximately 15% of human sera (unpublished data). In order to determine the specificity of LPV antibodies in human sera, we performed competitive inhibition assays on 42 LPV reactive human sera (Fig. 9). The LPV reactivity of 35 (83%) sera was inhibited by more than 50% by LPV VLPs and less than 50% by either BKV or JCV VLPs. Six (14%) sera were inhibited by more than 50% by LPV and BKV or JCV, and one serum sample (2%) was not inhibited by any VLP tested. These data indicate that LPV antibodies in human sera, unlike SV40 antibodies, cannot be attributed to cross reacting BKV or JCV antibodies.

Conclusions

We have demonstrated low levels of BKV and JCV antibodies in SV40 antibody positive macaques and low levels of SV40 antibodies in BKV or JCV antibody positive humans. In addition, we have shown that the BKV and JCV seroreactivity in macaques is competitively inhibited by the heterologous SV40 VLP and the SV40 seroreactivity in human sera is inhibited by heterologous BKV or JCV VLPs. Taken together the data suggest that SV40 infection

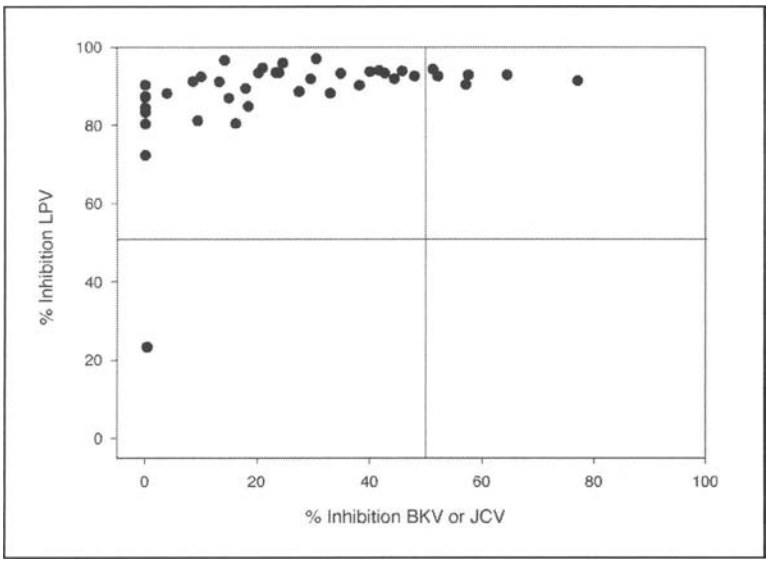

Figure 9. Scatter plot of percent competitive inhibition of LPV reactivity of 42 human sera by LPV and BKV or JCV VLP protein. Serum samples were tested in the LPV VLP EIA in the presence of 4ug/ml of LPV, BKV or JCV VLP protein. The assay was performed as described in the legend to Figure 3 and percent inhibition was calculated as described in the legend to Figure 4. The percent inhibition by LPV VLP protein (y-axis) is plotted versus maximal percent inhibition by BKV or JCV VLP protein (x-axis). The horizontal and vertical lines mark 50% inhibition by LPV and BKV or JCV VLP protein, respectively.

induces cross reacting antibodies to the human polyomaviruses and the human polyomaviruses, particularly BKV, induce cross reacting antibodies to SV40. The inhibition by a heterologous VLP cannot be explained by a shared antigen present in the VLP preparations because MPV and LPV VLPs did not inhibit the SV40 reactivity of human sera or the BKV and JCV reactivity of macaque sera. The serological cross reactivity is not surprising given the 77% amino acid identity between the JCV and SV40 VP1 proteins and the 83% identity between the BKV and SV40 VP1 proteins. However, JCV and BKV VP1 proteins, which share 80% amino acid identity, are antigenically distinct. Since BKV and JCV both infect humans, the most likely reason for the distinct antigenicity of their capsid proteins is positive selection for unique immunodominant surface epitopes, which allow one virus to replicate in the presence of immunity to the other. We identified a small number of SV40 antibody positive human sera that were inhibited by SV40 and not by BKV or JCV VLPs. Whether this reactivity is due to exposure of humans to SV40 needs to be addressed in carefully designed epidemiological studies and confirmed by detection of SV40 in biological samples from SV40 antibody positive subjects.

Lymphotropic polyomavirus was first described in 1979 and early serological studies using a variety of assay methods reported a seroprevalence of ~30% in human populations. Our studies with VLP-based assays confirm the presence of LPV reactive antibodies in a substantial proportion of human sera (~15%). Furthermore, competitive inhibition assays show that the reactivity is not the result of cross-reactivity with BKV or JCV antibodies. Although LPV was isolated from a B-lymphoblastoid cell line derived from an African green monkey, the host range of LPV is unknown. Takemoto et al[42]detected LPV antibodies in serum samples from humans and multiple species of nonhuman primates using an immunofluorescence assay. The highest seroprevalence was in gorillas (77%), Orangutans (58%), African green monkeys (48%) and squirrel monkeys (36%). In other species the seroprevalence ranged from 8-30% and in

humans was 27%. The antibody titers were not reported. Given the host species specificity of other polyomaviruses it is unlikely that all these species are infected with the same virus. Serosurveys using VLP-based assays together with attempts to detect LPV viral sequences by PCR should be performed to determine the host species for LPV. Perhaps the LPV reactivity of human sera and that of some nonhuman primates is due to cross reacting antibodies induced by unknown LPV-related polyomaviruseses.

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