CHARACTERIZATION OF THE BINDING AND NEUTRALIZING PROPERTIES OF MONOCLONAL ANTIBODIES AGAINST JCV

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ABSTRACT:

Background: Monoclonal antibody-based immunity to JC polyomavirus (JCV) is not well understood and monoclonal antibodies (mAbs) that functionally neutralize the infectivity of JCV have not been documented. Methodology: Recombinant JCV viruses (VLPs) were produced in a baculovirus system, and mouse mAbs against this protein generated using standard methods. Six mAbs that bound to VLPs in ELISA assays were obtained for further characterization. Results: Four antibodies recognized epitopes present only in intact VLPs, while the other two antibodies reacted only with denatured VLPs. Five of the six antibodies cross-reacted with VLPs based on JCV’s core relative BHV-1, the only JCV-specific mAb, clone JCV-10.13 (isotype IgG2a) recognized a conformational epitope. This mAb potently neutralized the infectivity of pseudocapsids based on wild-type JCV genotypes 1A and 2A, but neutralized pseudocapsids based on the distantly related JCV genotype 3B very poorly. JCV-10.13 was also neutralized the infectivity of a JCV PV strain from a patient with multifocal encephalopathy (buried epitopes). For virus neutralizing assays McAbs were tested as tissue culture supernates diluted 1:100. RESULTS: (1) VLP-based ELISAs can detect JCV-binding antibodies that do not necessarily neutralize the infectivity of JCV. These observations have relevance for developing VLP-based vaccines or therapeutic mAbs against JCV. VLPs from multiple genotypes may be needed to formulate a vaccine that could protect against diverse JCV strains circulating in patients with progressive multifocal encephalopathy (PME).

INTRODUCTION:
The human antibody response to JCV is incompletely understood and it is not clear why some patients develop PML, while others do not. There is also an unmet clinical need to develop therapies against PML. Over the past two decades, testing of small molecules as anti-JCV agents has not resulted in any notable success for patients with PML. Therefore, it is necessary to further explore the possibility of using neutralizing antibodies against JC virus in the prophylactic and immunotherapeutic setting. To date, limited attention has been paid to the notion of antibody neutralization of JCV. This is understandable, since the penetration of antibodies into the brain will be limited by the blood brain barrier. However, this issue needs to be revisited in the era of Nanomods which allows therapeutic agents to be effectively delivered to specific cell and tissue targets. Over and above their potential therapeutic use, epitope characterization and cross-recognition of neutralizing antibodies will facilitate an understanding of JCV humoral immunity and stimulate the development of vaccines.

METHODS:

BKV, JCV, and SV40 virus-like particles (VLPs) were prepared using standard methods (1-6). Hybridomas secreting monoclonal antibodies (McAbs) reactive to VLPs were generated. Monoclonal antibody reactivity against both intact and disrupted VLPs was assessed by virus-binding ELISA assays. Each McAb was reacting with intact but not disrupted pseudocapsids were interpreted as recognizing conformational epitopes located on the exposed surface of viral capsid (surface epitopes). Persistent or residual reactivity after denaturation was taken as evidence of linear epitopes. In case of reactivity with both intact and disrupted pseudocapsids, epitopes were assumed not to be exposed on the surface (buried epitopes). For virus neutralizing assays McAbs were tested as tissue culture supernates diluted 1:100. A volume of 500 ml was incubated with 100,000 infectious BK or SV40 virions per pseudovirion at 37 degrees C for 2 hours to neutralize VP-1 epitopes on the viral capsid protein. McAb treated and control viremia were then infected with 38 cells (BKV, ATCC VR-837) or CV-1 cells (SV40, Stokk #449). BKV neutralizing activity was assessed by measuring TK+ DNA load on day 7 of cultures maintained in 25 square cm flasks (5). A reduction in viral DNA yield of >75% compared to control cells was accepted as a criterion for McAb neutralizing activity. SV40 neutralizing activity was assessed in analogous experiments using quantitative RTPCR analysis of large T antigen transcripts following infection of cells with SV40 viruses (a gift from M.J. Tevethia, Pennsylvania State University). Results were normalized for levels of cellular TATA binding protein. Determination of JCV neutralizing activity was based on pseudovirion assays developed in the Buck Laboratory. Plots of % inhibition of Guassian keratocyte release versus log McAb concentration were used to calculate 50% (EC50) and 90% (EC90) neutralizing concentrations for each McAb. Antibodies were tested against a broad panel of JCV reporter/effector corresponding to various natural wild-type JCV genotypes (1A, 2A, and 3B), and as a reagentive set of McAb was used that detected the cerebrospinal fluid of PML patients (mutants GCO1, L595, N2655, S267F, S269F, and Q271K).

RESULTS:

Immunoassay determination of the six McAbs raised established that one each was IgG1 and IgG2, two were IgG3, and the remaining three were IgM. In ELISA-based binding assays the McAbs bound JCV VLP with mean OD ranging from 0.510 (JC-3.6) to 2.06 (JC-10.13). Of twelve McAbs to BKV and eleven McAbs to SV40 only one BKV antibody (BK F11) and 3 SV40 antibodies (VPI, F11, H12) bound weakly to JCV VLP (Figure 1). Thus, it is possible to raise species-specific McAbs to different JCV genotypes. JC-2.5, JC-3.6, JC-7.9, and JC-9.16 also showed cross binding to BKV pseudocapsids, while JC-3.6 and JC-9.16 also cross bound intact SV40 pseudocapsids (data not shown). JC-2.5, JC-3.6, JC-7.9, and JC-9.10 recognized intact pseudocapsids, presumably at exposed conformational epitopes. JC-7.9 and JC-9.16 recognized only denatured JCV, BKV, and SV40 pseudocapsids suggesting the existence of linear buried epitopes common to all 3 human polyomavirus species studied. The predominance of conformational epitopes with JCV-9.13 also neutralized the infectivity of a subset of J CV pseudovirions carrying capist protein mutations typical of those found in the cerebrospinal fluid of patients suffering from JCV-induced progressive multifocal encephalitis (PME). In contrast to JCV-10.13 another conformation-specific mAb, JCV-9.16, failed to neutralize any tested JCV variants.

CONCLUSIONS:

(1) VLP-based ELISAs can detect JCV-binding antibodies that do not necessarily neutralize the infectivity of JCV. These observations have relevance for developing VLP-based vaccines or therapeutic mAbs against JCV. VLPs from multiple genotypes may be needed to formulate a vaccine that could protect against diverse JCV strains circulating in patients with progressive multifocal encephalopathy (PME).