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

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ABSTRACT:
Background: 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.
Methods: Recombinant JCV virus-like particles (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 close relative BKV.The only JCV-specific mAb, clone JCV-10.13 (isotype IgG2a) recognized a conformational epitope. This mAb potently neutralized the infectivity of pseudoviruses based on wild-Type JCV genotypes 1A and 2A, but neutralized pseudoviruses based on the distantly related JCV genotype 3B very poorly. JCV-10.13 also neutralized the infectivity of a subset of JCV pseudoviruses carrying capsid protein mutations typical of those found in the cerebrospinal fluid of patients suffering from JCV-induced progressive multifocal leukoencephalopathy (PML). In contrast to JCV-10.13, another conformation-specific mAb, JCV-9.16,failed to neutralize any tested JCV variants.
Conclusions: These data show that VLP-based ELISAs can detect JCV-binding antibodies that do not detectably 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 PML. Functional neutralization-based serology will be required for effective validation of candidate JCV VLP vaccines and therapeutic mAbs.

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 JCV neutralizing antibodies as a potential immunoprophylaxis and immunotherapeutic option. To date , only limited attention has been paid to the notion of antibody-based therapies for 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 re-visited in the era of Nano-medicine which allows therapeutic agents to be effectively delivered to specific cell and tissue targets. Over and above their potential therapeutic use, epitope recognition and cross-recognition studies of a panel of JCV monoclonal 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. McAbs 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 disruption was taken as evidence of linear epitopes. In case of reactivity only to 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 1:1000. A volume of 5 µl was incubated with 100,000 infectious BK or SV40 virions pseudovirions at 37 degrees C for 2 hours to neutralize VP-1 epitopes on the viral protein capsid. McAb treated and control virions were then used to infect WI-38 cells (BKV, ATCC# VR 837) or CV-1 cells (SV40, Stock #449). BKV neutralizing activity was assessed by quantitating BKV 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 RT-PCR analysis of large Large T-antigen transcripts following infection of cells with SV40 virions (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 Gaussian luciferase release versus $\log_{10}$ 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 vectors corresponding to various natural "wild-type" JCV genotypes (1A, 2A, and 3B), and a a representative set of JCV mutant genotypes found in the cerebrospinal fluid of PML patients (mutants GCN1, L55F, N265S, S267F, S269F, and Q271K).

RESULTS: Immunoglobulin isotype determination of the six McAbs raised established that one each was IgG1 and IgG2, two were IgG2b,, while 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 (VP1- F11, H2, I2) bound weakly to JCV VLP (Figure 1). Thus, it is possible to raise species-specific McAbs to different polyomaviruses. JC-2.5, JC-3.6, JC 6.7, 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-9.16 and JC-10.13 recognized only intact pseudocapsids, presumably at exposed conformational epitopes. JC-6.7 and JC-7.9 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 has is similar to what has been observed in similar studies performed on another structurally similar virus, namely human papillomavirus (4). Conformational epitopes are generally more efficient in inducing a potent antibody mediated response than linear epitopes. Thus, it has been shown that papillomavirus capsomeres (pentameric subcapsid particles produced in E.coli) elicit lower antibody titers than intact VLP. On the other hand, papillomavirus capsids can be as efficient as intact VLP in evoking CD8 positive T-cells responses (7).

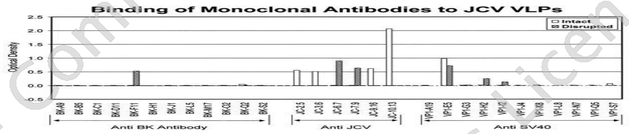

Figure 1: Binding of anti-BKV, anti-JCV and anti-SV40 monoclonal antibodies to JCV virus like particles (VLPs) evaluated by ELISA based binding assays. Optical density values plotted on the Y-axis are mean of duplicate values. Hatched bars represent binding of binding after disruption of VLPs in 0.2 M Na_2CO_3 buffer (pH 10.6) containing 0.01 M dithiothreitol.

The only JCV-specific monoclonal antibody clone JCV-10.13 (isotype IgG2a), recognized a conformational epitope. In the Buck Laboratory, this monoclonal antibody potently neutralized the infectivity of pseudoviruses based on wild-type JCV genotypes 1A and 2A, but neutralization of genotype 3B was poor (Figure 2). JCV-10.13 also neutralized the infectivity of a subset of JCV pseudoviruses carrying capsid protein mutations typical of those found in the cerebrospinal fluid of patients suffering from PML. In contrast to JCV-10.13, another conformation-specific mouse monoclonal antibody, JCV-9.16, failed to neutralize any tested JCV variants.

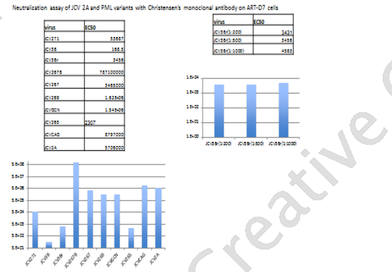

Figure 2: Neutralizing properties of McAb JCV-10.13. EC50 titers are presented numerically (top left) and as a bar graph (bottom left) for JCV genotypes 1A (JCVCAG), 2A, 3B, and stereotypical mutants described in the cerebrospinal fluid of patients with progressive multifocal encephalopathy (PML). It can be noted that JCV-10.13 neutralizes wild-type JCV genotypes 1A (JCVCAG) and 2A (JCV2A) with EC50 titers in the low millions, but neutralizes wild-type JCV genotype 3B (JCV3Br) with a titer in the low thousands. Results of testing against JCV 3Br are presented at 3 different concentrations, with a dlution as low as 1:100 ((top right and bottom right). With respect to PML mutants, 10.13 neutralizes PML variants in the 2A background (267, 269) but doesn't neutralize 265, which is in the 3B background..

CONCLUSIONS:
(1) VLP-based ELISAs can detect JCV-binding antibodies that do not necessarily neutralize the infectivity of JCV. Therefore, functional neutralization-based serology will be needed to validate candidate JCV VLP vaccines and therapeutic McAbs
(2) The neutralizing activity of McAbs can be specific for particular genotypes and clinical strains. Hence, 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. PML.

FUTURE PLANS:
Existing antibodies to JCV have all been raised in animals and cannot be evaluated for therapeutic efficacy, since there is no experimental mode for PML currently available. Therefore, we will seek to produce human JCV neutralizing monoclonal antibodies, which have the potential of being directly transferred to the clinical arena a without genetic engineering to eliminate xenoreactivity.

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