

# Amino acid sequences mediating vascular cell adhesion molecule 1 binding to integrin alpha 4: homologous DSP sequence found for JC polyoma VP1 coat protein

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## Abstract

The JC polyoma viral coat protein VP1 was analyzed for amino acid sequence homologies to the IDSP sequence which mediates binding of VLA-4 (integrin alpha 4) to vascular cell adhesion molecule 1. Although the full sequence was not found, a DSP sequence was located near the critical arginine residue linked to infectivity of the virus and binding to sialic acid containing molecules such as integrins (3). For the JC polyoma virus, a DSP sequence was found at residues 70, 71 and 72 with homology also noted for the mouse polyoma virus and SV40 virus. Three dimensional modeling of the VP1 molecule suggests that the DSP loop has an accessible site for interaction from the external side of the assembled viral capsid pentamer.

## Introduction

Administration of an antibody (natalizumab) to the alpha 4 integrin molecule binding site to the head region of the vascular cell adhesion molecule 1 (VCAM-1) molecule was found to be associated with systemic JC viremia and subsequent progressive multifocal leukoencephalopathy (PML) in a 60 year old Crohn's disease patient who had been previously treated with infliximab and azothioprine.<sup>1</sup> After only 6 administrations of the natalizumab antibody, serum JC viral load rose from being undetectable to 520 copies/mL and then to 6600 DNA copies/mL two months later when PML was suspected, and required brain biopsy for diagnosis; up to 3.9 million JC virus DNA copies per brain glial cell were found.

Since the exact cause of the JC viremia remains unclear in this case, the future therapeutic role for natalizumab in treating multiple sclerosis and other disorders remains uncertain. To help understand the pathogenesis of antibody related JC viremia and subsequent PML, it is hypothesized here that the alpha 4

integrin antibody may disrupt the peripheral binding of the virus in natural tissue reservoirs in certain individuals; JC viremia after blockade of the normal alpha 4 integrin binding sites for the virus in lymphoid tissue such as spleen and the tonsils could overwhelm the normal cytotoxic T cell immune surveillance defense mechanisms. Indirect support for this hypothesis of peripheral binding of VP1 to alpha 4 integrin in lymphoid tissue comes from the work of Caruso *et al.*<sup>2,3</sup> who found an important VP1 receptor function for the alpha 4 integrin molecule, which like the beta 1 integrin molecule is heavily glycosylated with terminal sialic acid residues,<sup>4</sup> which were already known to mediate VP1 attachment. This study therefore attempts to identify critical amino acid binding sequences that may mediate an interaction between alpha 4 integrin molecules in normal host tissue and the JC virus VP1 coat proteins. Since IDSP is a critical VCAM-1 amino acid sequence for binding to the alpha 4 integrin,<sup>5</sup> a search was made to find a similar sequence in the VP1 protein that could mediate an attachment to integrins.

## Materials and Methods

Using accession number J02288.1, the protein sequence for the polyoma VP1 coat protein was analyzed for IDSP sequences using the National Center for Biotechnology Information on-line data base (on-line web address: <http://www.ncbi.nlm.nih.gov>). Enumeration of the amino acid residues followed the convention of numbering VP1 residues after the initial Met residue (Caruso *et al.*<sup>3</sup>). Alignment information for different strains of polyoma virus was analyzed by a conserved domain search at the following on-line web address: <http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>.<sup>6</sup>

The localization of the DSP sequence for the human polyoma VP1 protein was studied using the Molecular Modeling Database (MMDB), which enables reconstruction and interactive viewing of the three dimensional macromolecular structures with Cn3D software; web address: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Structure>

The individual VP1 protein structure was analyzed as well as one of the 72 pentameric icosahedral capsids that cover the surface of the polyoma virus, and visualized in relation to hydrophobicity as well as charge.

## Findings

Although the full IDSP sequence was not found, a DSP sequence was found for the JC polyoma virus VP1 coat protein (accession number P03089) as well as Mouse polyomavirus (strain p16 small-plaque; accession-

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number 1SIE\_A), as well as Chain 1, Simian Virus 40 (accession number 1SVA\_1), but not for Kilham polyomavirus (accession number P24595), Budgerigar fledgling polyomavirus (BFPyV; accession number P13891), Hamster polyomavirus (HaPyV; accession number P03092), African green monkey polyomavirus (accession number P04010), or Bovine polyomavirus (accession number P24848). Alligned short segments of VP1 which display the homologous DSP sequence are shown in red in Figure 1 with residues 70,71, and 72 for the JC polyoma virus being aspartic acid (D), serine (S), and proline (P) respectively. The three dimensional external view of a single pentamer capsid (Figure 2) reveals accessible DSP residues for interaction and potential ligand binding. A detailed interactive review of a ball and stick type model (Figure 3) one of the five individual molecules that form a pentamer suggests a peripheral location to the DSP loop in relation to the bulk of the VP1 molecule, with the suggestion that a pocket is formed by this eccentric peripheral loop that could allow insertion of a small ligand molecule into this site.

## Discussion

As reported in preliminary form by Meyer,<sup>7</sup> a DSP sequence has now been found that may

represent a alpha 4 integrin binding site for the major JC polyoma virus external coat protein known as VP1. Since the IDSP sequence is critical in binding VCAM-1 to the alpha 4 integrin molecule, and since the alpha 4 integrin molecule is also thought to serve as a post-attachment receptor for VP1,<sup>2</sup> it is possible that that antibodies to the alpha 4 integrin molecule leaves the virus in peripheral lymphoid tissue in a free and unbound state. Since the alpha 4 integrin antibody natalizumab is associated with JC viremia and subsequent overwhelming brain infection by the virus (PML), it is hypothesized that alpha 4 integrin molecules in lymphoid tissue normally binds to the JC virus VP1 protein to render it non-infective by successfully competing with other receptors for the virus. The binding may be mediated by the DSP motif at residues 70,71, and 72 for JC, and at 88,89,90 for the murine polyoma A2 strain; for the latter, a nearby critical Arginine residue at position 77 governs infectivity of the virus and is believed to bind to sialic acid containing molecules such as integrins.<sup>3</sup> It is unclear if DSP mediated binding of VP1 to the alpha 4 integrin molecule produces secondary conformational changes at the nearby VP1 Arginine 77 sialic acid binding site to alter infectivity of the virus.

Extensive literature exists regarding other viruses that display specific binding to integrins; antibodies to specific integrins can prevent infection by specific cytopathic viruses that normally do not show the JC phenomenon of long term benign persistence and dormancy within tissue. The alpha 6 integrin likely serves as a cellular receptor for papillomaviruses,<sup>8</sup> whereas the alpha 5 beta 6 integrin binds to cocksackie virus A9 through a RGD amino acid sequence;<sup>9</sup> this sequence is also implicated in the binding of B cell lymphotropic polyoma virus capsids to the alpha beta 3 integrin.<sup>10</sup> When the alphav is found in combination with beta 8, this integrin can serve as a receptor for the foot and mouth disease virus;<sup>11</sup> when alphav combines with beta 1, the integrin molecule dimer can be a receptor for the adenovirus.<sup>12</sup> The alphav integrin is particularly important, as other combinations yield different viral specificities: when combined with beta 3, the integrin dimer can bind the West Nile virus,<sup>13</sup> cytomegalovirus,<sup>14</sup> as well as Rotavirus.<sup>15</sup> Regarding the Kaposi's sarcoma-associated herpesvirus, viral entry is mediated by binding to the alpha3beta1 integrin.<sup>16</sup> Beta 3 integrin has been found to serve a receptor function for the Hantavirus.<sup>17</sup> Infectivity seems to depend on binding to inactive, bent, alphavbeta3-integrin molecules at a specific bend site at the plexin-semaphorin-integrin (PSI),<sup>18</sup> where molecular motion could otherwise take place to straighten the molecule into an active form.

Further research is needed to understand if

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YYGWSRGINLATSDETDSPGNNTLPTWSMAKLQLPMLNEDLT 113
QKGLSKSLAAEKQFTDDSPDKEQLPCYSVARIPLPNINEDLT 103
FYGFSDNITVSADYTQDMPRIKELPCYSMAKISLPLNEDMT 101
KNGYSTVITVQAEGYQDAPHSTEVPCYSCARIPLPTINDDIT 91
YYGFSQS IKVNSSLTADEVKANQLPYYSMAKIQLPTLNEDLT 101
LYGYSNSINTAFSKASDTPNKDTLPCYSVAVIKLP LLNEDMT 104
YYGFSDNQRGSTSRTEDEDLISAELPRYSLGVVQLPLLNEKLT 103
LRGFSKSI S ISDTFESDSPNRDMLPCYSVARIPLPNINEDLT 96

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Figure 1. The sequence alignment (red displaying the DSP sequence) shows portions of the initial segment of amino acids for the following VP1 coat protein for polyoma viruses, in descending order from top down: Mouse polyomavirus (strain p16 small-plaque), Simian virus 40, Kilham polyomavirus, Budgerigar fledgling disease, polyomavirus (BFPyV), Hamster polyomavirus (HaPyV), African green monkey polyomavirus, Bovine polyomavirus, JC polyomavirus.

alpha 4 integrin binding to the DSP sequence of VP1 normally leaves the JC virus in a non-infective state. Successful competition with other tissue receptors for the virus might be an adequate explanation for why the JC virus can be found in normal tissue of over 70% of normal individuals without causing PML,<sup>19</sup> which is extremely rare in fully immunocompetent individuals, yet linked to PML in up to 4 to 6% of immunosuppressed HIV infected patients. In vitro studies are needed as well to test if synthetic DSP sequences can inhibit binding of VP1 to alpha 4 integrin molecules; should such an interaction be documented, it may have relevance to the understanding the natural biology of the JC polyoma virus, which can remain non-infective in not only kidney and lymphoid tissue, but also even the brain.<sup>19</sup> Further understanding of how the JC virus persists is important in understanding multiple disease states, including primary brain tumors, which have been found to harbor the virus and express T antigens in oligodendrogliomas.<sup>20</sup>

This study has limitations in that the key publications by Caruso *et al.*<sup>2,3</sup> which found a viral VP1 protein receptor function for the alpha 4 integrin molecule, derived their research findings from the murine polyoma virus; however, like the beta 1 integrin molecule and the serotonin receptor molecule, the alpha 4 integrin molecule is heavily glycosylated with terminal sialic acid residues. It is important to also note that blockade of the serotonin receptor 2A by risperidone does not inhibit JCV attachment, internalization and replication in primary human fetal glial cells,<sup>21</sup> raising questions about the biologic relevance about data concerning the serotonin receptor also serving as a proposed receptor for the JC virus in light of the well established role for integrin molecules as receptors for other viruses; furthermore, linear sialylated pentasaccharides with the sequence NeuNAc- $\alpha$ 2,6-Gal- $\beta$ 1,4-GlcNAc- $\beta$ 1,3-Gal- $\beta$ 1,4-Glc present on host

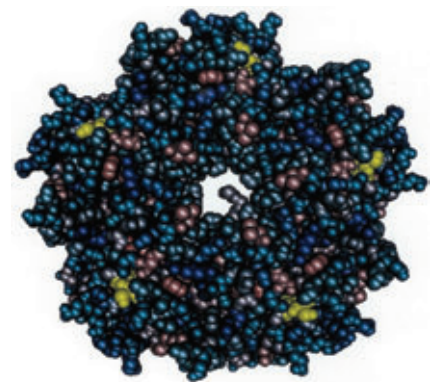


Figure 2. A view of the external face of a pentameric capsid of VP1 proteins (one of 72 capsids comprising the JC polyoma virus); the DSP sequence is seen in yellow for each of the five VP1 subunits.

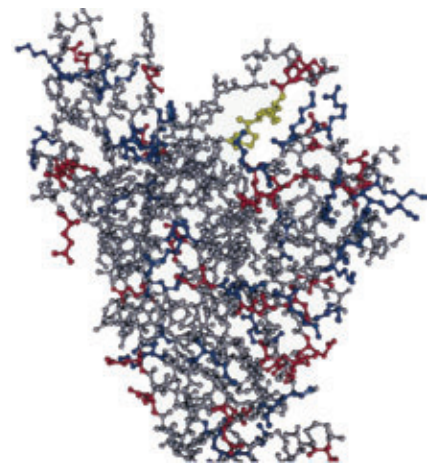


Figure 3. The VP1 protein as a ball and stick representation colored according to charge, with yellow indicating the DSP sequence homologous to the IDSP sequence mediating VCAM-1 binding to the alpha 4 integrin molecule.

glycoproteins and glycolipids have now been found to serve as a specific JCV recognition motif – future research is needed to understand how this applies to integrin alpha 4 terminal oligosaccharide sequences.<sup>22</sup>

Finally, it is important to point out that caution with the use of Natalizumab is strongly advised, as the latest data has revealed an incidence rate of 212 confirmed cases of PML among 99,571 patients treated with Natalizumab, equating to 2.1 cases per 1000 treated patients.<sup>23</sup>

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