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VARIABLE MAJOR PROTEINS OF *BORRELLIA HERMSII*

Epitope Mapping and Partial Sequence Analysis of CNBr Peptides

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A strategy used by some pathogenic microorganisms for survival in their hosts is antigenic variation (1, 2). *Borrelia hermsii*, an etiologic agent of relapsing fever, is a bacterium capable of manifesting 26 or more serotypes during the course of infection (3, 4). The spontaneous switching from one antigen type to another occurs at a rate estimated to be $10^{-4}$ to $10^{-3}$ per cell per generation (3). Although antigenic variation by *B. hermsii* appears to be independent of the immune response, it is possible that host immunity or other factors play some role in determining which serotypes predominate in a given situation (3). Previous studies from our laboratory (5, 6) showed that antigenic variation is associated with change in a single, abundant, surface-exposed protein, referred to as a pl protein, which is hereafter designated as a variable major protein (VMP). The VMPs of all serotypes of *B. hermsii* HS1 examined to date differed in their apparent molecular weights, their peptide maps, and their reactivities with serotype-specific polyclonal and monoclonal antibodies (4-6). In many respects, the phenomenology of antigenic variation in *B. hermsii* resembles that of the salivarian trypanosomes (7-9).

Our efforts are toward understanding the molecular mechanisms used by *B. hermsii*, and probably other relapsing fever borreliae, to vary their surface antigens. The present analysis of VMP structure was designed to determine whether serotype-specific epitopes are confined to limited regions of the proteins and to further assess the degree of structural relatedness between VMP. We now report on two borrelial VMP that were isolated, fragmented, mapped with antibodies, and partially sequenced. The results indicate that VMP are the products of genes belonging to a polygene family.

Materials and Methods

Organisms and Culture Conditions. The origin of the HS1 strain (ATCC 35209) of *B. hermsii* and the isolation of the isogenic serotypes C, 7, and 21 of this strain have been...
described previously (3–5). The borreliae were cultivated in mice and broth medium essentially as described previously (6). Exceptions were an x radiation dosage per mouse of 650 instead of 900 rad and the use of BSK II medium (10) for the broth cultivations. Cultures were harvested, assessed by immunofluorescence as to homogeneity of serotype, and stored frozen as described previously (5, 6).

Production and Screening of Hybridomas. For the majority of hybridomas reported here, the mouse immunization schedule was as follows: frozen suspensions of spirochetes were thawed, centrifuged, washed twice, and finally resuspended in phosphate-buffered saline, pH 7.4, with 5 mM MgCl₂ (PBS/Mg). BALB/c mice (6 wk old) were each inoculated intraperitoneally with \( \sim 10^8 \) borreliae on day 0. On day 3, after peak spirochtemia and clearance of the infecting spirochetes had occurred, the mice was begun on oral tetracycline hydrochloride (0.5 mg/ml of drinking water) to prevent emergence of new serotypes. The antibiotic was continued until day 13. On day 21, the mice were inoculated intravenously with the same inoculum and same serotype of borreliae as on day 0. On day 24, the mice were sacrificed and their spleens removed. The remaining steps in the production and assessment of hybridomas, through fusions of the spleen cells with derivatives of NS1 myeloma cells, have been described (5). The hybridomas were usually cloned by limiting dilution, but in some cases cloning was done in semisolid medium by the method of Davis et al. (11).

In the alternative immunization protocol, a spirochete suspension in PBS/Mg was emulsified in Freund's complete adjuvant and injected intraperitoneally into mice on day 0. The spirochete inocula were \( \sim 10^6 \) per mouse. The mice were boosted with live organisms on day 21 and sacrificed on day 24 as described above.

The supernatant fluids of the hybridomas were screened by indirect immunofluorescence assay (IFA) (5, 12) and also by a solid phase radioimmunoassay (RIA) (13). In a modification of this RIA method for use with whole cells, 10 μl of a suspension of spirochetes in PBS/Mg (\( \sim 10^9 \) cells/ml) was applied to 6.4-mm-diam discs of Whatman 540 filter paper (Whatman, Inc., Clifton, NJ). The discs were dried at 42°C, fixed for 30 min in methanol, dried again, and stored at 4°C. In the assay itself the discs were placed in flat-bottomed microtiter plate wells, blocked with a solution of bovine albumin, incubated with hybridoma supernatant, washed, and probed for bound immunoglobulin G with radiiodinated protein A, as described previously (13).

Detergent Extraction of VMP. Frozen cell suspensions containing \( \sim 5 \times 10^{10} \) spirochetes were thawed and centrifuged at 10,000 g for 20 min. The pellet was washed in 30 ml of TSEA (50 mM Tris, pH 7.6, 150 mM NaCl, 5 mM EDTA, and 0.05% sodium azide). After another centrifugation, the second pellet was resuspended in 20 ml of a lysis-and-solubilization solution consisting of 2% octylglucopyranoside (Calbiochem-Behring Corp., La Jolla, CA) and 50 mM dithiothreitol in TSEA. After incubation of the suspension in a water bath at 55°C for 1 h, the lysis mixture was centrifuged at 20,000 g for 60 min at 35°C. The supernatant was dialyzed against 2 l of distilled water at 4°C; there were three to four changes of the water over 48 h. The precipitate that formed was isolated by centrifugation at 10,000 g for 5 min.

High Performance Liquid Chromatography (HPLC). Isolation of the VMP was accomplished on a Waters HPLC equipped with model U6K injector, data module, model 720 system controller, and a model 450 variable wavelength detector set at 220 nM (Waters Associates, Milford, MA). The microfuge pellet was dissolved in 0.5 ml anhydrous trifluoroacetic acid (TFA) (Sequanal grade; Pierce Chemical Co., Rockford, IL) and chromatographed using a reverse phase gradient, 1 × 25 cm Vydac C-4 column (The Separations Group, Hesperia, CA). Buffer B was 0.1% TFA in water; buffer B was 0.1% TFA, 67% acetonitrile, 33% isopropanol (Burdick & Jackson Laboratories, Muskegon, MI). For the preparative isolation of VMP, a linear gradient of 20–100% buffer B was run at a flow rate of 3 ml/min for 30 min. Half-minute fractions were collected by connecting a fraction collector to the outlet of the detector.

The separation of the cyanogen bromide (CNBr) fragments (see below) was accomplished in a similar fashion except using a 0.4 × 25 cm Vydac C-4 column, a flow rate of
1 ml/min, and a gradient of 0–100% buffer B. Injection volumes for the CNBr fragment varied from 80 to 100 µl.

**CNBr Cleavage of the VMP.** ~1 mg of purified VMP was solubilized in 1 ml 70% formic acid (14). 50 µl of acetonitrile containing 2 mg/ml CNBr (Eastman Kodak Co., Rochester, NY) was added, and the solution was incubated for 24 h at room temperature. The reaction mixture was then dried in a Speed-Vac (Savant Instruments, Inc., Hicksville, NY) and solubilized in TFA for injection into the HPLC.

**Polyacrylamide Gel Electrophoresis (PAGE) and Western Blot Analysis.** PAGE was performed as described previously (5, 6); and the ratio of acrylamide to bis-acrylamide was 30:0.8. The pH of the separating gel was 8.6. HPLC samples were dried in a Speed-Vac before solubilization in the sample buffer. Prestained high and low range molecular weight standards (Nos. 6020 and 6021) were obtained from Bethesda Research Laboratories, Bethesda, MD. Western blot analysis was performed as described previously (12).

**Automated Sequence Analysis.** In most cases, automated Edman degradation was performed on an Applied Biosystems, Inc. (Foster City, CA) model 470A protein sequencer with program MHTFA (see Operator’s Manual) as previously described (15). In some cases, sequence analyses were performed on a Beckman 890C sequencer (Beckman Instruments, Inc., Fullerton, CA) with cold trap and autoconversion modifications.

**Results**

**Monoclonal Antibodies.** A Coomassie Blue-stained PAGE gel of serotypes C, 7, and 21 is shown in Fig. 1. The apparent subunit molecular weights of the VMP of the three serotypes were 21, 36, and 35 × 10³, respectively, when their
migrations were compared with those of molecular weight standards. The other major protein in the lysates had an apparent molecular weight of $37 \times 10^3$ in each of the serotypes. This latter, invariable protein has been designated pII (5). The electrophoretic migrations of the VMP relative to the pII protein and also to each other vary with the pH of the separating gel buffer and also the amount of crosslinking of acrylamide in the gel (5, 6).

A previous study (5) showed that polyclonal and monoclonal antibodies that were serotype-specific as assessed by immunofluorescence bound only to the homologous VMP in Western blots. The IgM monoclonal antibodies used were derived from spleens removed during the clearance of the infecting spirochetes. We have since raised IgG monoclonal antibodies by boosting the mice with a second dose of the homologous serotype 3 wk after the initial infection. Tetra-cycline administration prevented relapses with other serotypes between the two immunizations.

In most cases of hybridomas derived from "natural" infections, i.e., those in which the initial immunization material was live organisms injected into the peritoneum without an adjuvant, the resultant monoclonal antibodies were serotype specific by IFA, RIA, and Western blots. These reactions were exemplified by antibodies H12534 and H10211 (Fig. 1 and Table I). Some monoclonal antibodies, e.g., H12125 in Table I, were serotype specific by IFA and RIA but did bind to VMP (or any other component) in Western blots.

We found a single monoclonal antibody, H12123, that bound to more than one but not all serotypes. This antibody recognized serotypes 7 and 21 by IFA and RIA (Table I) but not serotype C (Table I) or serotypes 3, 14, or 17 (data not shown). H12123 bound to both VMP$_7$ and VMP$_{21}$ in Western blots (Fig. 1 and Table I). It was the product of a hybridoma derived from a mouse immunized with serotype 7 organisms in complete Freund's adjuvant. Although such an immunization cannot be considered one that mimicked a "natural" infection, the

| TABLE I |
| Reactivities of Monoclonal Antibodies Against Whole Cells, Intact VMPs, and CNBr Peptides of VMP$_7$ and VMP$_{21}$ |

| Monoclonal antibody | Whole cell RIA* | Western blot |
|---------------------|----------------|--------------|
|                     |                | Reactivities of antibody |          |
|                     |                |                   |          |
|                     |                | Intact VMP | CNBr peptides | VMP$_7$ | VMP$_{21}$ |
|                     |                | C 7 21 | 16 K 20 K 7 K 14 K 14.5 K |
| H12125              | - + -         | - - - | ND |
| H12534              | - + -         | - - - | ND |
| H9733               | - + -         | - - - | ND |
| H12936              | - + -         | - - - | ND |
| H12123              | - + +         | + + + | + - - |
| H10211              | - - +         | - - - | ND |
| H10022              | - - +         | - - - | ND |
| H4116               | - + +         | - - - | ND |

* See Materials and Methods.

* Not determined.
results with this antibody suggest that VMP$_7$ and VMP$_{21}$ share regions of structure in common.

**Purification of VMP.** The borrelial cells were lysed immediately by the octyl-
glycopyranoside detergent solution. Heating to 55°C produced an opacification of the lysate solution; the precipitate was removed by centrifugation. The supernatant was enriched for VMPs, but it also contained large quantities of material with an ultraviolet absorption spectrum that was consistent with nucleic acids (data not shown). Dialysis of the detergent supernatant against water precipitated the VMP and a few other minor proteins while leaving the nucleic acid-like material in solution. The crude, VMP-containing precipitate was recovered by centrifugation. A comparison of the octyl-glucopyranoside extraction pellet (OGP) and supernatant (OGS) is shown in Fig. 2. The pII proteins and most other cell proteins were in the OGP fraction; there was enrichment of VMP$_7$ and VMP$_{21}$ in the OGS fraction.

The crude VMP material recovered from the OGS was insoluble in buffers A and B of the reverse phase system, but it was soluble in anhydrous TFA. Once loaded onto the C4 reverse phase HPLC column, the VMP remained sufficiently soluble and eluted in a highly hydrophobic portion of the HPLC gradient, i.e., ~90% buffer B. The elution profile for VMP$_{21}$ is shown in Fig. 3. Similar profiles were obtained during chromatography of OGS material from serotypes 7 and C (data not shown).

**CNBr Fragments.** The HPLC fractions containing VMP$_C$, VMP$_7$, and VMP$_{21}$ were reacted with CNBr in 70% formic acid. Results with 70% TFA were comparable. There was no apparent cleavage of any VMP upon incubation in formic acid or TFA alone (data not shown). CNBr cleaved VMP$_7$ and VMP$_{21}$ but not VMP$_C$.

The CNBr fragments of VMP$_7$ and VMP$_{21}$ were recovered by reverse phase HPLC using an analytical column and a buffer B gradient of 0–100% for better resolution.

![Figure 2](image-url)
HPLC purification of *B. hermsii* VMP<sub>7</sub>. Octyl-glucopyranoside soluble fraction from *B. hermsii* was solubilized in 0.5 ml TFA and chromatographed on a 1 × 25 cm C-4 column using a reverse phase gradient of 20–100% buffer B over 30 min. Flow rate was 3 ml/min, and 1.5-ml fractions were collected. OD<sub>280</sub> was monitored during the separation using an OD absorbance unit, full scale sensitivity. VMP<sub>7</sub> was recovered from fractions 54–57.

resolution of small fragments (Fig. 4). The chromatography fractions corresponding to the peaks in the eluates were subjected to PAGE. Peaks A and B of CNBr-treated VMP<sub>7</sub>, and peaks C, D, and E of CNBr-treated VMP<sub>21</sub>, contained Coomassie Blue-stainable components. The small OD<sub>220</sub> peaks at fractions 20–23 and 56–59 of the CNBr-VMP<sub>21</sub> (Fig. 4) eluate contained no Coomassie Blue-detectable proteins when analyzed by PAGE.

The electrophoretic migrations of the recovered CNBr fragments in relation to those of uncleaved VMP<sub>7</sub> and VMP<sub>21</sub> are shown in Fig. 5. The two CNBr peptides of VMP<sub>7</sub> had apparent molecular weights of 16,000 (16 K) and 20 K and together probably account for the whole of this VMP. The isolated CNBr fragments of VMP<sub>21</sub> had apparent molecular weights of 7, 14, and 14.5 K.

Reactivities of Monoclonal Antibodies with CNBr Fragments. We used both the CNBr reaction mixtures and the HPLC-isolated peptides to determine the binding patterns of several monoclonal antibodies in Western blots. Examples of antibody reactions in blots are shown in Fig. 6. H9733 bound to intact VMP<sub>7</sub> and to the 20-K CNBr fragment of VMP<sub>7</sub>. The anti-serotype 21 antibody, H4116, reacted with VMP<sub>21</sub> and its 14.5 K mol wt CNBr peptide. The minor bands with molecular weights greater than the major band probably represent antibodies binding to partial cleavage products in the mixtures. Table I, in addition to listing the reactivities of antibodies to whole cells and to intact VMP, also gives the specificities of representative antibodies for the different CNBr peptides. One antibody, H12524, bound to VMP<sub>7</sub> but not to either of the CNBr fragments. The remaining Western blot-positive antibodies listed in Table I bound to a CNBr fragment. We did not find an antibody that bound to more
than one CNBr peptide of given VMP. Each of the five CNBr fragments we isolated was recognized by at least one serotype-specific antibody.

Antibody H12123, which reacted in IFA, RIA, and Western blot with whole cells and intact VMP of serotypes 7 and 21, bound to the 16 K mol wt fragment of VMP, and the 14 K mol wt fragment of VMP, (Table I). Although these
FIGURE 5. PAGE of HPLC-purified VMP7 and VMP21 and HPLC fractions of CNBr diges-
tions. HPLC was performed on the octyl-glycopyranoside extracts of serotypes 7 and 21 as
described in the text and demonstrated in Fig. 3. Fractions containing VMP7 or VMP21 were
pooled and dried. The dried proteins were resolubilized in sample buffer and subjected to
PAGE. Fraction pools A and B of the CNBr digestion of VMP7 and fraction pools C, D, and
E of the CNBr digestion of VMP21 were recovered and analyzed by PAGE in the same way.
The 15% acrylamide gel was stained with Coomassie Blue. The apparent molecular weights
of the major bands in each fraction are indicated.

FIGURE 6. Western blot analyses of reactivities of monoclonal antibodies H9733 (left) and
H4116 (right) against intact VMP and CNBr digestion mixtures. Extracted VMP7 (left) and
VMP21 (right) were incubated in formic acid alone (–) or with CNBr (+) as described in the
text. After being dried, the samples were subjected to PAGE and then to Western blot analysis.
The gel contained 15% acrylamide. The apparent molecular weights of the major band in
each CNBr digest are indicated.
results indicate that the two VMP under study shared an epitope and possibly a region of amino acid sequence, there were other antibodies, e.g., H12136 and H10211, that bound to one but not both of these particular CNBr fragments. Thus, there were serotype-specific epitopes even on the fragments that contained a common epitope.

**Partial Amino Acid Sequence Determination.** Initial attempts to determine the N-terminal amino acid sequences of whole VMPc, VMP7, and VMP21 by either vapor phase or spinning cup protein sequencing procedures were unsuccessful. We concluded that the N-termini of these proteins were blocked and, therefore, proceeded with sequence analysis of the CNBr fragments of VMP7 and VMP21. Although VMPc was not cleaved by CNBr, it seemed possible that a modified N-terminal methionine on VMPc might be removed during treatment, thus allowing sequencing. However, this did not occur with CNBr-treated VMPc.

The N-terminal sequences from the five CNBr fragments of VMP7 and VMP21 are shown in Fig. 7. Sequence analysis of cleaved but un fractionated VMP7 and VMP21 yielded two, in the case of VMP7, or three, in the case of VMP21, amino acids at each position. This represented a combination of the individual sequences from each VMP and indicated that additional CNBr fragments were not present in the mixtures.

As there were varying degrees of homology between the fragments, we arbitrarily aligned in pairs four of the five sequences: VMP7 (20 K mol wt) with VMP21 (14.5 K), and VMP7 (16 K) with VMP21 (14 K). Knowing the site of cleavage for CNBr, we also assumed that a methionyl residue preceded the N-terminal residue of each CNBr fragment’s sequence.

Sequence alignment revealed homologies of approximately 80 and 40% for the pairs VMP7(20 K)–VMP21(14.5 K) and VMP7(16 K)–VMP21(14 K), respectively. Although the VMP21(7 K) fragment showed little homology with any of

![Figure 7](#)

**Figure 7.** Amino acid sequence analysis of the N-termini of the CNBr fragments of VMP7 and VMP21. The five HPLC-purified CNBr fragments shown in Fig. 4 are identified by the VMP from which they were derived and by their apparent molecular weights. Automated sequence analysis was performed as described in the text. Single letter abbreviations are as follows: A, alanine; D, aspartic acid; E, glutamic acid; F, phenyl alanine; G, glycine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. Fragments are aligned to provide maximum homology. The boxes indicate regions of homology between pair members. Sequences common to VMP7 (20 K) and VMP21 (7 K) are underlined.
the other four sequence, the sequence S-A-E-X-A-X-X-A-X-X-E was present in both VMP\(_7\)(20 K), beginning with residue 5, and VMP\(_{21}\)(7 K), beginning with residue 2. A closely related sequence, S-A-E-X-A-X-S-X-X-E, was found in VMP\(_{21}\)(14.5 K). None of the intervening “X” amino acids in these similar sequences were charged. However, the residue before the initial serine was charged in each case: an arginine in VMP\(_7\)(20 K), a lysine in VMP\(_{21}\)(14.5 K), and an aspartic acid in VMP\(_{21}\)(7 K).

Discussion

The results confirmed our earlier assessment that VMP differ in their antigens and their overall structures (4–6). The assortment of reactivities obtained with an expanded battery of monoclonal antibodies and the patterns produced by CNBr cleavage were consistent with past findings. Nonetheless, as we focused nearer to the VMP molecules, we found local similarities as well as dissimilarities between VMPs.

The first indication of structural conservation was the binding of monoclonal antibody H12123 to whole cells and isolated VMP of both serotypes 7 and 21. The accessibility of the binding site for H12123 in the native state, i.e., live organisms in a host or culture medium, is not known. Studies to determine the cross-neutralization potential of the monoclonal antibodies are in progress. It is possible that the presentation of spirochetes in an oil emulsion and the fixation of the cells in methanol allowed processing or exposure of antigens seldom seen during natural infections. For the purposes of the present study, however, antigenic crossreactivity was demonstrated, and we proceeded to examine the structure of these VMP more closely.

The abundance of VMP among the spirochetes’ proteins and the differences in solubility in the detergent octyl-glycopyranoside between the VMP and the other major cellular protein, pII, aided VMP extraction. The elution of the VMP at high concentrations of acetonitrile during HPLC and the subsequent difficulty in solubilizing the VMP in aqueous solutions indicated that, in this respect, these proteins are unlike the more readily solubilized surface glycoproteins of trypanosomes (16).

The blockage of the VMPs’ N-termini necessitated fragmentation of the proteins before further analysis. Although the deduced sizes for the five CNBr peptides could account for the entire lengths of VMP\(_7\) and VMP\(_{21}\), we cannot completely exclude the existence of small fragments that escaped our detection and recovery efforts. A hypothetical missing peptide could explain the binding in Western blots of antibody H12524 to intact VMP\(_7\) but not to either of the two recovered CNBr fragments of VMP\(_7\). Alternatively, the epitope for H12524 could span the two peptides.

Other monoclonal antibodies did recognize CNBr fragments in blots. Indeed, for each peptide, there were antibodies that bound to it only, with the exception of H12123. Serotype-specific epitopes were not limited to one part of the proteins. There is, therefore, still no evidence in VMP of a lengthy and continuous constant region as occurs in immunoglobulins (17–19) and gonococcal pili (20–22).

Although there may not be extensive uninterrupted regions of homology
between VMP, the results with antibody H12123 and, subsequently, of amino acid sequencing, indicated that there are short regions of primary structure shared between VMP7 and VMP21. The pair of CNBr peptides, VMP7(16 K) and VMP21(14 K) had only 40% homology in their N-terminal sequences, but they also contained the H12123 epitope that was common to VMP7 and VMP21, presumably downstream toward their C-termini.

Application of Hopp and Woods' algorithm (23), which predicts relative hydrophobicity and hydrophilicity, to the N-terminal sequences indicated that the known portions of VMP7(20 K) and VMP21(14.5 K) are comparatively hydrophobic. In contrast, the N-termini of VMP7(16 K) and VMP21(14 K) are hydrophilic and thus probably solvent exposed. The location of the highest Hopp and Woods value, +1.6, was between residue 8 and 9 in VMP7(16 K). This is in the region of greatest divergence between the aligned sequences of VMP7(16 K) and VMP21(14.5 K).

Of the four residues that differed between VMP7(20 K) and VMP21(14.5 K), all replacements could have been the consequence of only a single base change in each of the codons. In contrast, of the eight residues differing between VMP7(16 K) and VMP21(14 K), one-half the substitutions would have required no fewer than two base changes in each of the codons. The evolutionary distance, which is defined as the number of nucleotide substitutions per length of DNA sequence, between the N-termini of VMP7(16 K) and VMP21(14 K) is greater than that implied by a nondiscriminatory accounting of amino acid replacements.

Although our picture of borrelial VMP structure is incomplete, it is becoming clearer. VMPs differ operationally in their antigens but two VMPs can have regions of partial amino acid homology. It seems likely, therefore, that these proteins belong to one or more polygene families (24), as do the variable surface glycoproteins of trypanosomes (25). The family members have highly polymorphic regions that appear to be solvent exposed. We would predict that those other parts of the VMP molecule with greater conservation of amino sequence are, in general, relatively hydrophobic and are under evolutionary pressure to retain certain structural features.

As we learn more about VMP structure and the molecular mechanisms governing their differential expression, we should be able to study again the pertinent mammals and arthropods and examine in greater detail the finely tuned interplay between parasite, host, and vector.

Summary

The variable major proteins (VMP) of serotypes 7 and 21 of the relapsing fever agent *Borrelia hermsii* were isolated by detergent extraction and high performance liquid chromatography. Cyanogen bromide (CNBr) digestion of the isolated VMP yielded two peptides of apparent molecular weights 20,000 (20 K) and 16 K from VMP7, and three peptides of 14.5, 14, and 7 K mol wt from VMP21. Serotype-specific monoclonal antibodies bound in Western blots to one of each of the two or three CNBr fragments from the homologous VMP. A single monoclonal antibody bound to the whole cells, the isolated VMP, and a CNBr fragment of both serotype 7 and serotype 21. (This crossreactive antibody did not, however, bind to any of the four other serotypes examined.) Regional
conservation of structure between VMP\textsubscript{7} and VMP\textsubscript{1} was also shown by amino acid sequence analysis of the N-termini of the CNBr fragments. One pair of aligned fragments from VMP\textsubscript{7} and VMP\textsubscript{1} had 80\% amino acid homology in sequence; a second pair had 40\% homology. The partial amino acid homologies between two VMP suggest that these proteins are products of members of a polygene family.

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