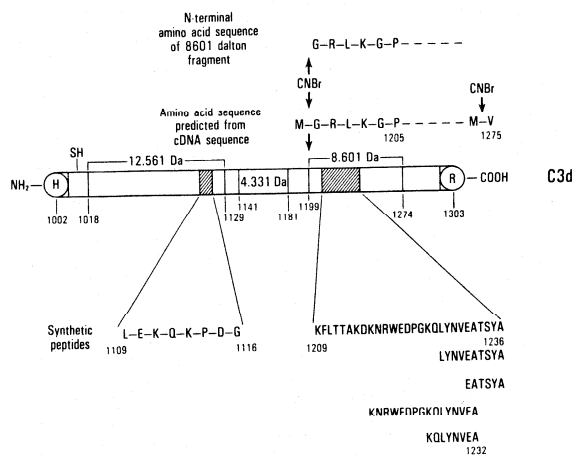


SYNTHETIC PEPTIDES PREDICTED FROM THE NUCLEOTIDE SEQUENCE OF C3 BIND TO C3d RECEPTOR AND MONOCLONAL ANTIBODY 130

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The C3d fragment of C3 contains the domain that binds to the C3d receptor (CR<sub>2</sub>) expressed on B lymphocytes which also serves as a receptor for Epstein Barr Virus (EBV) (1). C3d also contains a neoantigenic determinant recognized by MoAb 130 which is expressed when C3b is cleaved to iC3b and subsequently to C3dg or C3d. The MoAb 130 inhibits the binding of C3d to its receptor. By using isolated C3d fragments and synthetic peptides constructed according to the primary structure of C3d, the C3d receptor binding site and the MoAb 130 antigenic site within the C3d fragment were localized.

(I) Characterization of the C3d fragment generated by CNBr. Five different fragments were isolated by HPLC following fragmentation of C3d by CNBr. Analysis of the different fragments by ELISA employing MoAb 130 revealed that only the 8,601 dalton fragment contained the antigenic determinant recognized by this antibody. In addition, by using two different assay systems, a) binding of microsphere bound C3d fragments to Raji cells and b) binding of <sup>125</sup>I-gp72 (2) to C3d fragments fixed to microtiter



plates, it was found that the same 8,601 dalton C3d fragment contained the C3d receptor binding site. N-terminal amino acid sequence analysis of the 8,601 dalton fragment and comparison of this sequence with the amino acid sequence deduced from the cDNA sequence (3) placed the 8,601 dalton fragment between amino acids 1199 and 1274 of the C3 sequence (Figure 1).

Figure 1. Positioning of the different C3d fragments is based on N-terminal amino acid sequence, methionine cleavage sites and comparison with the amino acid predicted from cDNA sequence.

(II) Synthetic peptides. In order to further localize the binding site for the C3d receptor and for MoAb 130 within the 8,601 dalton fragment, six

synthetic peptides (1209-1236, 1227-1236, 1217-1232, 1225-1232, 1109-1116) predicted from the areas of C3d with greatest hydrophilicity were synthesized (Figure 1). Table 1 shows the binding of MoAb 130 to synthetic peptides and the inhibition of binding of  $^{125}\text{I}$  synthetic peptide (1209-1236) to Raji cells by the different synthetic peptides. Based on these results and the comparison of the amino acid sequence predicted by the known cDNA sequence of mouse and human C3 (4), it is concluded that the binding site for CR<sub>2</sub> is located within amino acid sequence 1225-1232 and the MoAb 130 antigenic site within amino acid sequence 1217-1225.

Table 1

REACTIVITY OF MoAb 130 AND CR<sub>2</sub> WITH SYNTHETIC PEPTIDES

Synthetic Peptides	Number of Residues	Binding to MoAb 130	Inhibition of binding of $^{125}\text{I}$ Peptide (1209-1236) to CR <sub>2</sub>
1109-1116	8	—	—
1209-1236	28	+	+
1227-1236	10	—	+
1217-1232	16	+	+
1225-1232	8	—	+
1231-1236	6	—	—
C3d		+	+
C3c		—	—

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