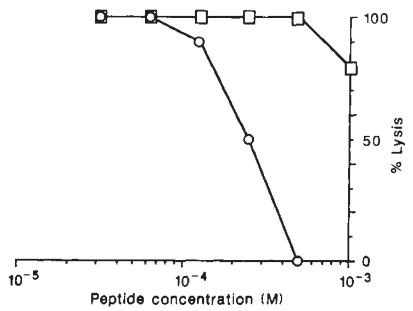

Fig. 3 Inhibition of complement lysis by synthetic peptides. Circles mark lysis for the peptide AcAla-Glu-Ala-Lys-Ala-Lys-Ala-NH_2 (based on the motif), and squares mark a control peptide Ser-Asp-Glu-Lys-Ala-Ser-Pro-Asp-Lys-Tyr, which includes both acidic and basic residues.

Methods. Lysis of sheep red blood cells was as in Fig. 1. A chequerboard assay (ref 28: mouse IgG2b antibody titrated in the first dimension and guinea pig complement source in the second) was used to determine the lowest antibody concentration giving complete lysis after 30 min incubation. Peptide was purified by reverse phase HPLC and the composition checked by amino acid analysis. Freeze dried peptide was weighed and dissolved in CFD to a concentration of 4mM and serial dilutions of the peptide made into 200 μl NIP-derivatized red cells (5×10^7 cells per ml), guinea pig serum (1/40 dilution) and antibody (7 μg ml^{-1}), and incubated and scanned as in Fig. 1. An apparent I_{50} value of 170 μM compares with an I_{50} value of 75 μM (or 150 μM per CH_2 domain) reported for monomeric IgG[18].

In conclusion we have identified a C1q binding motif, Glu318, Lys320, Lys322 in the CH2 domain of the antibody, and a simple peptide based on this sequence is able to inhibit complement lysis. The charged nature of the C1q binding motif, the requirement for basic residues at 320 and 322, the ionic strength dependence of C1q binding, and the peptide inhibition data all suggest an electrostatic interaction. There are charged regions in each of the three chains in C1q heads, for example the sequence Glu 198-X-Asp 200-Y-Lys 202 in the A chain[21] which might interact with the motif as an antiparallel β strand. The C1q binding motif is a common sequence found in many proteins, and we suggest that recognition of this common motif in an array (for example in viruses and bacteria) directly by the multiple heads of C1q is an ancient defence mechanism. For example, retroviruses can be lysed directly by C1q in the absence of antibody[22]. Later in evolution, the antibody may have appeared as an adaptor, avoiding the requirement for the motif to be written directly on the pathogen.

We thank D. R. Burton and M. S. Neuberger for discussions, M. Bruggemann and H. Waldmann for the sequences of rat isotypes in advance of publication and R. Valerio for the peptides.

Table 1 Affinity of human C1q for mutant mouse IgG2b antibodies

Mutant	Lysis	Affinity for C1q (nM)
Asn 297 → Ala	−	31
Glu 318 → Ala	−	>300
Glu 318 → Thr	+	11
Glu 318 → Val	−	110
Lys 320 → Ala	−	>300
Lys 320 → Arg	+	11
Lys 320 → Gln	−	13
Lys 322 → Ala	−	>300
Lys 322 → Arg	+	11
Lys 322 → Gln	−	>300
wild type	+	11

The affinities of the mutants Glu 235 → Leu, Ile 253 → Ala, Gln 290 → Ala, Pro 331 → Gly, Glu 333 → Ala, Thr 335 → Ala and Ser 337 → Ala for C1q were also determined and lie in the range 9-12nM. A 10% suspension of Affigel 701 (Bio-Rad) polyacrylamide beads were derivatized with 4-hydroxy-3-iodo-5-nitrophenacetyl-ε-amino-*n*-caproic acid succinimide ester (Cambridge Research Biochemicals, UK) in 0.125 M sodium carbonate, at 37 °C in the dark for 24 h. Excess reagents were removed by successive centrifugation and decantation, and the NIP-cap-Affigel support stored as a 7% suspension in PBS containing 0.02% sodium azide at 4 °C in the dark. The support had a capacity for mouse IgG2b of about 10 mg ml^{-1}. The C1q binding assay used ^{125}I-labelled human C1q and was based on ref. 29.

Received 8 February; accepted 15 March, 1988.

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CORRIGENDUM

Multiple potassium-channel components are produced by alternative splicing at the *shaker* locus in *Drosophila*

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Nature **331,** 137-142

In Fig. 3*b* in this letter, a base was missed in the sequence of ShD1. An additional G should be present as the 48th nucleotide from the 5′ end. Thus the correct sequence beginning at nucleotide 44 should read: GAGCGGCTGC. By shifting the reading frame of the extreme 5′ end, a stop codon is introduced at nucleotides 42-44. Thus one or more of the five AUG codons that are present in this splicing variant and were identified as potential translational start sites are likely to serve this function and the predicted ShD protein is likely to be of approximately the same size as the other predicted products of the locus.

In addition, in Fig. 1 the locations of the mutations Sh^{K82a} and $T(1;3)Sh^{LC}$ were inadvertently shifted 5 kb to the left of the positions to which we had mapped them[1]. They still fall within the same intron as shown. A second graphics error has shifted the location of intron 11 in Fig. 3*a* one nucleotide to the left of its correct position.

Finally, the authors regret having omitted a reference to Goldman *et al.*[2] who demonstrated that a gene for a subunit of a putative neuronal nicotinic acetylcholine receptor is alternatively spliced. Thus alternative splicing may be a mechanism by which diversity is produced for many receptors or channels in both vertebrates and invertebrates.

The authors thank Dr Olaf Pongs for pointing out the sequence error.

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