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Homophilic adhesion mediated by the neural cell adhesion molecule involves multiple immunoglobulin domains

(recombinant proteins/FluoSpheres/neurons/antibodies)

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ABSTRACT **The neural cell adhesion molecule (N-CAM) mediates homophilic binding between a variety of cell types including neurons, neurons and glia, and neurons and muscle cells. The mechanism by which N-CAM on one cell interacts with N-CAM on another, however, is unknown. Attempts to identify which of the five immunoglobulin-like domains (Ig I–V) and the two fibronectin type III repeats (Fn_{III} 1-2) in the extracellular region of N-CAM are involved in this process have led to ambiguous results. We have generated soluble recombinant proteins corresponding to each of the individual immunoglobulin domains and the combined Fn_{III} 1-2 and prepared polyclonal antibodies specific for each. The purified proteins and antibodies were used in aggregation experiments with fluorescent microspheres and chicken embryo brain cells to determine possible contributions of each domain to homophilic adhesion. The recombinant domains were tested for their ability to bind to purified native N-CAM, to bind to each other, and to inhibit the aggregation of N-CAM on microspheres and the aggregation of neuronal cells. Each of the immunoglobulin domains bound to N-CAM, and in solution all of the immunoglobulin domains inhibited the aggregation of N-CAM-coated microspheres. Soluble Ig II, Ig III, and Ig IV inhibited neuronal aggregation; antibodies against whole N-CAM, the Ig III domain, and the Ig I domain all strongly inhibited neuronal aggregation, as well as the aggregation of N-CAM-coated microspheres. Of all the domains, the third immunoglobulin domain alone demonstrated the ability to self-aggregate, whereas Ig I bound to Ig V and Ig II bound to Ig IV. The combined Fn_{III} 1-2 exhibited a slight ability to self-aggregate but did not bind to any of the immunoglobulin-like domains. These results suggest that N-CAM–N-CAM binding involves all five immunoglobulin domains and prompt the hypothesis that in homophilic cell–cell binding mediated by N-CAM these domains may interact pairwise in an antiparallel orientation.**

The neural cell adhesion molecule (N-CAM) was the first cell adhesion molecule to be characterized extensively, and it provided the basis for the analysis of a number of different cell adhesion molecules in a variety of tissues of both vertebrates and invertebrates (1). N-CAM contains five immunoglobulin-like domains (Ig I–V) and two fibronectin type III repeats (Fn_{III} 1–2) and is expressed in a variety of alternatively spliced forms (2–4). There are two transmembrane forms (ld and sd) of N-CAM and one that is phosphatidylinositol-linked; evidence for a soluble form has also been obtained (5–7). N-CAM undergoes a number of posttranslational modifications including the developmentally regulated addition of large amounts of α-2,8-linked polysialic acid (2, 8–10).

N-CAM is expressed in a defined spatiotemporal pattern during embryonic development and is involved in a variety of neural cell interactions (11–14). N-CAM mediates homophilic binding between cells that express it; consistent with this homophilic mechanism, lipid vesicles reconstituted with purified N-CAM aggregate (15). When nonaggregating cells that normally do not express N-CAM are transfected with N-CAM cDNA (16–18), they also aggregate in an N-CAM-dependent manner.

Despite the considerable evidence for N-CAM homophilic binding, little is known about specific details of the binding mechanism. There have been a number of efforts to identify which domains are involved in the process. Early studies using monoclonal antibodies and N-CAM fragments indicated that binding involved Ig I–IV (2, 19, 20) and particularly Ig I–II and Ig III (2); the fifth immunoglobulin domain, which contains the polysialic acid, could influence binding but did not appear to be essential (19). Subsequent studies in other laboratories have differed in their conclusions. A recent study using chimeric molecules containing domains of N-CAM and those of carcinoembryonic antigen transfected into cells indicated that all five immunoglobulin domains are essential for N-CAM-mediated adhesion (17). This study, however, did not reveal which of the domains specifically interact with the others. Another transfection study using only N-CAM constructs indicated that mutations in Ig III had a significant effect on N-CAM binding and showed that peptides corresponding to a short region of Ig III could inhibit adhesion (18, 21, 22). In contrast, other studies of mouse N-CAM domains coated on plastic as substrates indicated that Ig I and II and the combined Fn_{III} 1–2 region each bound neuronal cell bodies, but Ig III had little, if any, activity in this type of assay (23).

To explore further which domains can participate in homophilic binding, we have generated the individual immunoglobulin domains and the combined Fn_{III} 1-2 of chicken N-CAM as soluble recombinant proteins, purified each to homogeneity, and tested them for binding when coated on fluorescent microspheres. All five immunoglobulin domains bound to N-CAM, and, in solution, each of the immunoglobulin domains inhibited N-CAM binding. Tests of interactions of individual domains with each other indicated that Ig I bound to Ig V, Ig II bound to Ig IV, and Ig III bound strongly to itself. In addition, neuronal cell aggregation could be inhibited by the addition of soluble Ig III and to a lesser extent by the addition of Ig II and Ig IV; Ig I, Ig V, and Fn_{III} 1–2 inhibited neuronal aggregation only modestly. The combined results indicate that all five immunoglobulin domains of N-CAM participate in binding and that the prominent role of Ig III probably derives from its ability to bind to itself. They also raise the possibility that in the intact N-CAM these domains interact on apposed cells in an antiparallel fashion.

MATERIALS AND METHODS

Proteins. Constructs for expressing the individual N-CAM domains were prepared by PCR from cDNA clones pEC208

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Abbreviations: N-CAM, neural cell adhesion molecule; endo-N, endo-*N*-acetylneuraminidase; Fn_{III}, fibronectin type III repeat(s); Ig I–V, immunoglobulin-like domains I–V, respectively; E, embryonic day.

and pEC254 (2) and cloned into the *Nco* I and *Bam*HI sites of pET-3d (Novagen). The nucleotide sequence of each construct was confirmed. The proteins were expressed in *Escherichia coli* BL21(DE3) and the recombinant proteins were purified from the bacterial sonicate as follows. The 40–70% $(NH_4)_2SO_4$ fraction of Ig I was resuspended and serially purified over Sephadex G-75, SP-Sepharose, Sephadex G-75, and Sephadex G-50 (Pharmacia). The 40–70% $(NH_4)_2SO_4$ fraction of Ig II was purified over Sephacryl S-100, SP-Sepharose, and Sephadex G-50. The 40–70% $(NH_4)_2SO_4$ fraction of Ig III was purified over Sephacryl S-100, an N-CAM monoclonal antibody 1 affinity column, and Sephadex G-50. The sonication pellets of Ig IV and Ig V were resuspended in 50 mM diethylamine (pH 11.5), neutralized, and passed over a DE-52 column. Purity of the preparations was tested by SDS/PAGE and immunoblot analysis.

Polyclonal antibodies to individual domains and Fab′ fragments were prepared as described (11). The ability of the polyclonal antibodies to recognize endo-*N*-acetylneuraminidase (endo-N) treated N-CAM was assayed by spotting equivalent amounts of N-CAM on nitrocellulose, incubating with antibodies and then with ^{125}I-labeled protein A, and exposing the blot in a PhosphorImager (Molecular Dynamics); results were quantitated using IMAGEQUANT software.

N-CAM was purified from embryonic day (E) 12–14 chicken brain membranes (24); treatment with endo-N (gift of Frederic A. Troy, University of California, Davis) was for 2 hr at 37°C in 50 mM Tris (pH 8.0) followed by gel filtration on Sephadex G-25 to remove residual sialyl oligomers. Protein concentrations for all samples were determined using the Bio-Rad DC protein assay.

FluoSpheres. Proteins were coupled to 0.6-μm amidine-modified fluorescent latex microspheres (FluoSpheres; Molecular Probes) by passive adsorption. FluoSpheres were washed and resuspended in 20 mM Tris (pH 8.0), added to protein solutions (0.5–1 mg/ml) to a final concentration of 0.5% solids, and gently agitated for 3 hr at 20°C. The microspheres were resuspended in 20 mM Tris, pH 8/1 mg of bovine serum albumin per ml, incubated for 1 hr at 20°C to block unreacted sites, washed, and resuspended to their original volume in phosphate-buffered saline (PBS)/0.1 mg of bovine serum albumin per ml.

Coated microspheres were sonicated (15 sec), mixed at a ratio of 1:4 with PBS/0.1 mg of bovine serum albumin per ml, incubated for 1.5 hr at room temperature without agitation, and then 20-μl samples were diluted in 10 ml of Isoton II. The appearance of superthreshold particles was monitored by using a Coulter Counter fitted with a 70-μm aperture (K = 1.46) set at amplification = ½, aperture current = ½, threshold = 20–100. These settings allowed detection of particles >7.3 μm^3, equivalent to an aggregate of 64 microspheres. Pretreatment of microspheres with Fab′ was performed at 4°C for 30 min.

Neuron Aggregation Assays. E8 chicken embryo brain cells were prepared as described (25). Aggregation assays were performed in 24-well dishes with 3×10^5 cells per ml in Hanks' balanced salt solution/20 mM Hepes, pH 7.5/2 mM EDTA. Samples were rotated (100 rpm) at 37°C for 30 min, fixed with an equal volume of 2% glutaraldehyde, diluted in 10 ml of Isoton II, and counted in a Coulter Counter. Inhibition was determined as a percentage decrease in particle number. Pretreatment of cells with proteins or Fab′ was performed at 4°C for 30 min. Aggregation is measured as the disappearance of single neurons; percent aggregation = (sample aggregation/control aggregation) × 100, with aggregation calculated as 1 − (sample counts/control counts).

RESULTS

To analyze the contributions of the various domains to homophilic adhesion, a series of nonoverlapping proteins that span the entire extracellular region of N-CAM was made (Fig. 1*A*). PCR primers were designed so that the only expressed bacterial sequence was the initiator methionine plus one amino acid necessary to preserve the reading frame. The regions between the immunoglobulin loops were divided evenly between two contiguous domains, and the sequences were designed to be contiguous from domain to domain with no overlap, with two exceptions: (*i*) to produce Ig IV, the amino terminus of the construct was extended 3 amino acids into Ig III (Phe-Ala-Lys) to enhance solubility; and (*ii*) to produce Ig V, the amino terminus was extended 6 amino acids into Ig IV (Tyr-Leu-Glu-Val-Gln-Tyr) to enhance induction in the bacterial expression system.

The proteins were purified (Fig. 1*B*) and the identity of each protein was confirmed by Western blotting with polyclonal anti-N-CAM antibodies and by amino-terminal sequence analysis. On SDS/PAGE (Fig. 1*B*), the proteins corresponding to domains III, IV, and V migrated more slowly than predicted, but mass spectrometry of Ig III and IV gave the predicted mass, so we assume that the mobility differences of Ig III, IV, and V on SDS/PAGE is an anomaly intrinsic to their sequences and ability to bind SDS.

Polyclonal antibodies were raised to each of the purified fragments. The antibodies were specific for the domain used as the immunogen and did not crossreact with the other domains (data not shown). The antibodies recognized purified N-CAM on Western blots but differentially bound native N-CAM on dot blots in the relative amounts: anti-Ig I, 100%; anti-Ig III, 74%; anti-Fn_{III} 1–2, 61%; anti-Ig IV, 21%; anti-Ig V, 15%; and anti-Ig II, 12%. It is not clear whether these differences result from folding of the N-CAM molecule, posttranslational modifications of N-CAM, or other effects. The purified domains were also used to map the epitopes of N-CAM monoclonal antibodies 1, 2, and 11. In accord with previous results (2, 19), antibody 1 bound only Ig III; antibodies 2 and 11 each bound only Ig I.

To avoid modification of the amino groups of N-CAM (26), we used microspheres that utilize passive adsorption (FluoSpheres). N-CAM attached to amidine FluoSpheres aggregated, and the aggregation was significantly enhanced by treatment with endo-N (27) (Fig. 2*A*). The results could be quantitated by measuring the appearance of superthreshold aggregates on a Coulter Counter (Fig. 3*A*); control FluoSpheres on which bovine serum albumin was adsorbed showed no significant aggregation. The appearance of N-CAM microsphere aggregates was blocked by the addition of Fab′ fragments of polyclonal antibodies to N-CAM and by monoclonal antibodies 1, 2, and 11. Antibodies to recombinant domains Ig I and Ig III also inhibited, whereas antibodies to the other

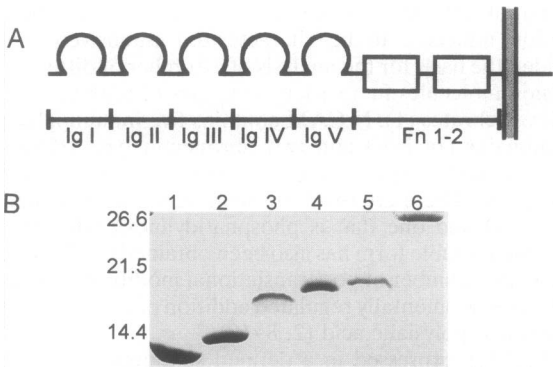

FIG. 1. Recombinant N-CAM domains. (*A*) Schematic diagram of the structure of the N-CAM molecule indicating the borders of the recombinant domains used in binding studies. (*B*) Coomassie blue stain of the purified N-CAM domains on a SDS/15% polyacrylamide gel. Lanes: 1, Ig I (10.9 kDa); 2, Ig II (10.9 kDa); 3, Ig III (10.5 kDa); 4, Ig IV (11.3 kDa); 5, Ig V (11.6 kDa); and 6, Fn_{III} 1–2 (23.1 kDa).

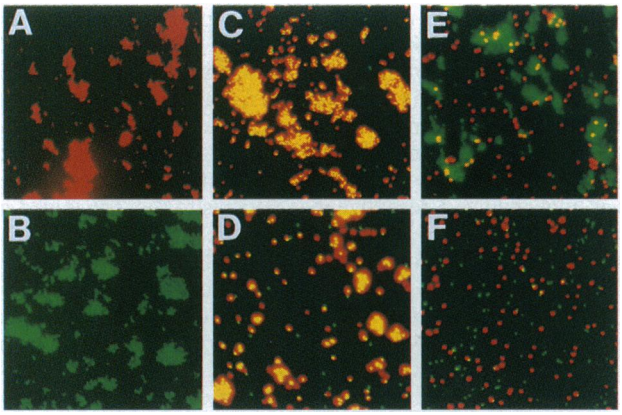

FIG. 2. Aggregation of protein-coated fluorescent FluoSpheres. (*A*) FluoSpheres coated with endo-N-treated chicken N-CAM formed large aggregates of the red beads. (*B*) Microspheres (green) coated with Ig III also formed large aggregates. (*C*) A mixture of Ig I-coated (red) and Ig V-coated (green) microspheres yielded mixed (yellow) aggregates, and (*D*) a mixture of Ig II-coated (red) and Ig IV-coated (green) microspheres also gave mixed (yellow) aggregates. (*E*) Ig III (green) microspheres aggregated strongly with themselves, but the aggregates did not incorporate Ig I (red) microspheres that were present; yellow dots appear where aggregates of Ig III overlap randomly distributed beads of Ig I. (*F*) Ig I (green) microspheres did not aggregate with Ig II (red).

domains had little effect, in accord with their relative ability to bind to intact N-CAM; increasing the concentrations of the antibodies to compensate for the difference in their reactivity with N-CAM led to nonspecific inhibition of the bead aggregation. Soluble recombinant domains were tested for their

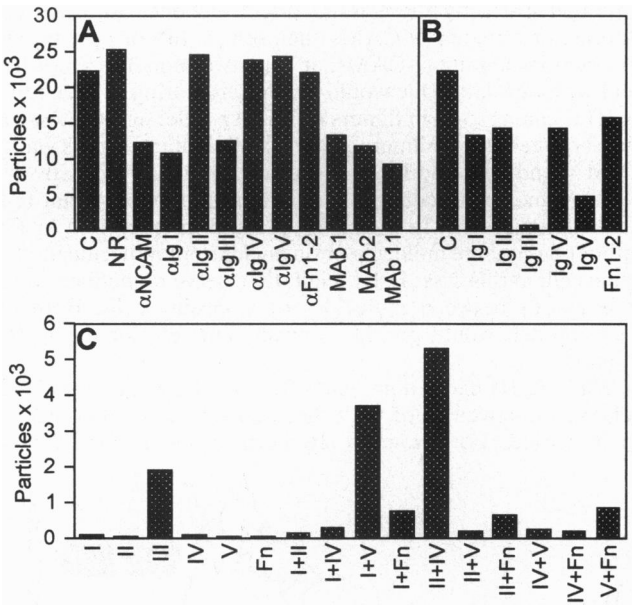

FIG. 3. Aggregation of N-CAM and recombinant domains on coated microspheres. (*A*) Aggregation of N-CAM-coated microspheres and inhibition of aggregation by Fab′ fragments (0.25 mg/ml) of antibodies specific to individual domains (e.g., αIg I) and monoclonal antibodies (mAb) 1, 2, and 11 (0.1 mg/ml). (*B*) Inhibition of aggregation of N-CAM-coated microspheres by soluble recombinant domains (0.25–0.5 mg/ml). (*C*) Aggregation of microspheres coated with individual recombinant N-CAM domains and tested, individually and in combinations (e.g., Ig I-coated microspheres plus Ig V-coated microspheres). Aggregation was measured as the appearance of superthreshold particles (≥60 beads). C, no additions; NR, addition of Fab′ from normal rabbit immunoglobulin (0.25 mg/ml); in all cases, N-CAM had been treated with endo-N.

ability to inhibit the aggregation of N-CAM-coated microspheres (Fig. 3*B*). Ig III was most effective followed by Ig V. All of the other domains had a moderate effect.

The ability of the individual domains to mediate aggregation was examined in the microsphere binding assay (Fig. 2 *B–F* and 3*C*). The Ig III domain was the only region that exhibited significant self-aggregation (Fig. 2*B*), although in some experiments, Ig IV was able to form aggregates when coated on the microspheres at high concentrations. Fn_{III} 1–2 did consistently form self-aggregates (data not shown), but in all cases the aggregates were very small (6–10 beads) and seldom reached the size of the superthreshold particles detected in the Coulter Counter (≥60 beads). When coated on beads, each of the immunoglobulin domains, but not Fn_{III} 1–2, aggregated with N-CAM-coated beads (data not shown).

In experiments examining combinations of domains, reproducible aggregation was demonstrated between Ig I and Ig V (Figs. 2*C* and 3*C*) and between Ig II and Ig IV (Figs. 2*D* and 3*C*). The apparent low levels of aggregation of Fn_{III} 1–2 with Ig I, Ig II, and Ig V (Fig. 3*C*) reflected small aggregates of Fn_{III} 1–2 only; in these mixtures Fn_{III} 1–2 aggregates were visualized in the microscope as small clusters of green microspheres, but red microspheres coated with Ig I, Ig II, or Ig V were not included in the clusters.

Of all the interactions, the aggregation of Ig III (Fig. 2*B*) was the most robust in that it formed more large aggregates than did the combinations of Ig I with Ig V (Fig. 2*C*) or Ig II with Ig IV (Fig. 2*D*). In general, aggregates of endo-N-treated N-CAM microspheres were the largest, followed by aggregates of Ig III, Ig I with Ig V, and then Ig II with Ig IV. Because of this difference in aggregate size, the data in Fig. 3*C* cannot be used rigorously as a quantitative measure of the relative strength of aggregation. Potential interactions between Ig III and the other domains could not be measured quantitatively in the Coulter Counter due to the inherent ability of Ig III to self-aggregate. Visual examination of mixtures of Ig III with the other domains, however, indicated that Ig III did not bind to any of the other domains (see Fig. 2*E*).

To analyze further the specificity of the binding of Ig I to Ig V, Ig II to Ig IV, and Ig III to itself, Fab′ fragments of antibodies to each recombinant domain were used as inhibitors (Fig. 4). Only antibodies specific for the domains coated on the bead were able to act as inhibitors. The results are in accord with the notion that these interactions are specific and that together they may all contribute to the basis for N-CAM homophilic binding.

The role of N-CAM domains was further investigated in neuron–neuron aggregation assays (Fig. 5). In accord with our previous studies (2, 19), antibodies to N-CAM and monoclonal antibodies 1 (Ig III), 2, and 11 (Ig I) all inhibited the aggregation of chicken embryo brain cells (Fig. 5*A*). Polyclonal antibodies to recombinant Ig I and Ig III domains strongly inhibited neuronal aggregation, but antibodies to the other recombinant domains had only marginal effects in accord with their ability to inhibit aggregation of N-CAM on microspheres (Fig. 3*A*). As anticipated, preincubation of neurons with soluble Ig III led to substantial inhibition of aggregation (Fig. 5*B*). Preincubation with soluble Ig II or Ig IV produced moderate inhibition, whereas preincubation with Ig I, Ig V, and Fn_{III} 1–2 had only weak effects.

DISCUSSION

Given its diverse influences on neural development and its potential role in regeneration, it is important to describe the mechanism of N-CAM binding, both to understand the processes influenced by N-CAM and to gain some insight as to how other molecules, including the polysialic acid on N-CAM, modulate N-CAM binding. Equally important, knowledge of the binding mechanism would allow the design of new specific

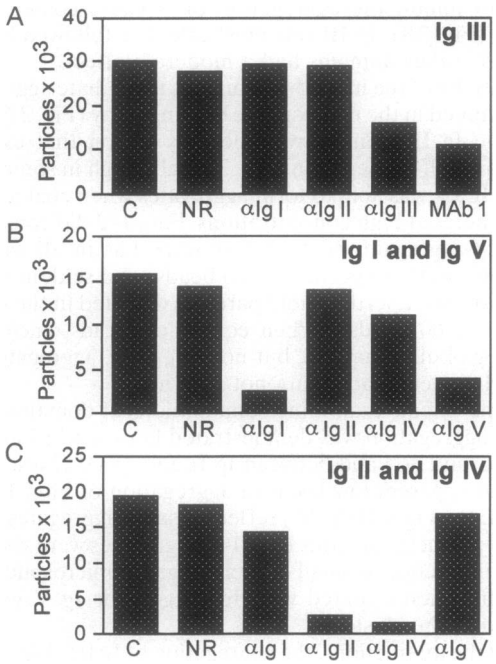

FIG. 4. Inhibition of aggregation of microspheres coated with immunoglobulin domains using Fab′ fragments from domain-specific antibodies. (*A*) Ig III aggregates. (*B*) Mixed aggregates of Ig II-coated microspheres and Ig IV-coated microspheres. (*C*) Mixed aggregates of Ig I-coated microspheres and Ig V-coated microspheres. Fab′ fragments were added to a final concentration of 0.25 mg/ml. C, no additions; NR, Fab′ from normal rabbit immunoglobulin.

reagents to perturb N-CAM binding. The results we present here indicate that all five immunoglobulin domains are involved in binding. One arrangement consistent with the data involves pairing of the domains in an antiparallel alignment, although other arrangements are not excluded.

The observation that all five domains can participate in N-CAM binding is consistent with earlier results (2, 19, 20) and agrees with recent studies of cells transfected with cDNAs encoding chimeras of N-CAM and carcinoembryonic antigen immunoglobulin domains. Our data are also in accord with a variety of results (2, 18, 19, 21) that suggest that Ig III has a special role in N-CAM binding and indicate that this effect is due to the ability of Ig III to interact with itself. Our results and those from cellular transfection studies (17, 18, 21, 22) differ

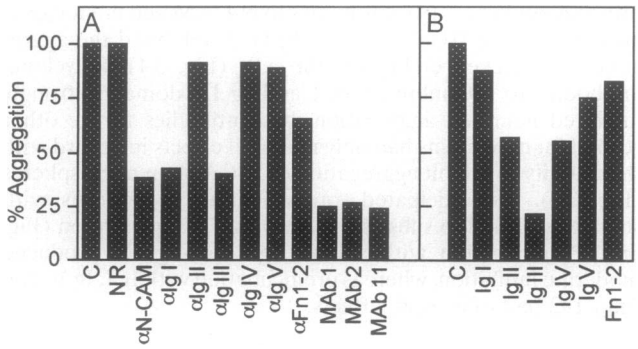

FIG. 5. Inhibition of neural cell aggregation using immunoglobulin domains, Fab′ fragments from domain-specific antibodies, and monoclonal antibodies. (*A*) Inhibition of neural aggregation by preincubation with soluble N-CAM domains (0.5 mg/ml). (*B*) Inhibition of neuronal aggregation by preincubation with Fab′ fragments (1 mg/ml) of antibodies to recombinant domains (e.g., αIg I) and monoclonal antibodies (mAb; 0.5 mg/ml) to N-CAM. The results are expressed as percent aggregation because this assay, unlike those with microspheres, measured aggregation as the disappearance of single neurons.

from a recent report (23) using partially purified recombinant proteins corresponding to the domains of mouse N-CAM. That study used the domains coated on plastic surfaces as substrates to examine neuron attachment and neurite outgrowth (23) and found that Ig I, Ig II, and Fn_{III} 1–2 allowed attachment of neuronal cell bodies, but Ig III had little effect. Attachment of neurons to N-CAM-coated substrates, however, may involve alternative mechanisms to those involved in N-CAM homophilic binding between cells; these alternative mechanisms may involve proteoglycans, for example (28–31). In accord with this notion, we have found in preliminary studies that when each of our recombinant domains was coated as a substrate, only Ig II, which binds proteoglycans (28), supported neuron attachment.

N-CAM–N-CAM interactions may occur between molecules on apposing cells (trans) or between molecules on the same cell (cis). Our main concern is with trans interactions that lead directly to cell–cell binding. The simplest model for trans binding suggested by our results is shown in Fig. 6, which depicts an N-CAM molecule on apposing cells aligned antiparallel so that the immunoglobulin domains are paired I with V, II with IV, and III with III. The bend between Ig IV and Ig V reflects the flexible hinge shown in electron micrographs to be between Ig III and Ig V (32, 33), and the polysialic acid on three potential sites on Ig V (9, 34) is indicated. The model shown is highly simplified; N-CAM binding is strongly concentration-dependent (15) and influenced by the polysialic acid, suggesting that the overall mechanism is more complex and involves additional interactions. For the model shown in Fig. 6, higher order aggregates of N-CAM could be formed if the alternatively aligned pairs interact with other pairs so that multiple molecules interdigitate in a zipper-like fashion.

Alternative models are possible, particularly if N-CAM molecules on the same cell can interact with each other, although presently there is no direct evidence for such cis interactions among N-CAMs themselves. In considering cis interactions among N-CAMs, at least two models are consistent with our data. One would involve cis pairing between the Ig III domains to form dimers (or higher order aggregates) on the same cell; these dimers, in turn, could interact trans via Ig I and V and Ig II and IV as suggested in Fig. 6. Alternatively, N-CAM molecules could fold into a hairpin loop, so that Ig I could interact with Ig V and Ig II could interact with Ig IV either on the same molecule or on an adjacent molecule on the same cell; in this case, Ig III might then serve to mediate trans interactions between molecules on apposing cells. Both of these models would provide a prominent role for the Ig III domain.

While Ig III had a dominant effect in all of our assays, the relative effectiveness of the other domains and antibodies to them varied. For example, Ig I on beads formed larger

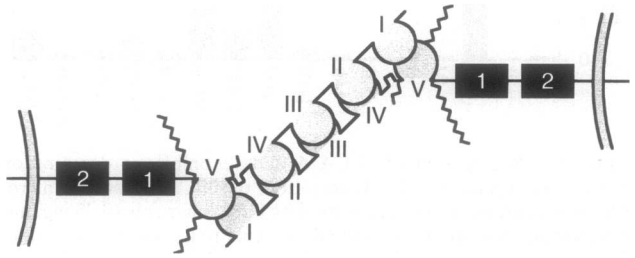

FIG. 6. Schematic of antiparallel model for N-CAM homophilic binding. Individual N-CAM molecules on apposing cells are aligned so that the immunoglobulin domains can pair I with V, II with IV, and III with III. The bend (∪) reflects the hinge region detected in electron micrographs of N-CAM molecules (32, 33) at an undetermined position in the region between Ig III and Ig V; the three stair step symbols of different lengths denote the polysialic acid of various amounts on three sites in Ig V (9, 34).

aggregates with Ig V on beads than Ig II on beads did with Ig IV on beads, but soluble Ig II and Ig IV were better inhibitors of neuronal aggregation. These variations may be due to glycosylation of N-CAM on the cell; for example, the weak effects of soluble Ig I and Ig V on neuron aggregation may be due to inhibition by the polysialic acid on Ig V in the N-CAM molecule *in vivo*. In all of our microsphere assays, the polysialic acid was removed from N-CAM. The influence of this highly charged sugar on the binding by individual domains, particularly on the potential interactions of Ig I with Ig V, clearly requires more extensive investigation.

There is no evidence that the carbohydrates play a direct role in N-CAM binding, although the ability of polysialic acid to modulate binding (15) is prominent. It should be pointed out that none of our recombinant domains is glycosylated whereas Ig III, IV, and V are probably glycosylated in the intact molecule *in vivo* (2, 9). Moreover, as previously mentioned, our general results are in accord with the cellular transfection studies using chimeric proteins of N-CAM and carcinoembryonic antigen; presumably all of the immunoglobulin domains in these studies were glycosylated. As indicated above, a short segment in Ig II binds heparin sulfate proteoglycans (28). However, the role, if any, of this interaction in N-CAM–N-CAM binding is unclear (29), and binding of N-CAM to chondroitin sulfate proteoglycans has been reported to inhibit cell adhesion (31). In addition, it has been suggested (35) that Ig IV can bind oligomannosidic glycans on L1, another member of the N-CAM family, and thus enhance L1-mediated cell adhesion. However, it is clear that none of these interactions is required for N-CAM homophilic binding.

Overall, our results support the notion that all five immunoglobulin domains are involved in N-CAM binding. In addition, they are consistent with the hypothesis that the domains in the intact molecule can align in an antiparallel fashion. Understanding the detailed mechanism of N-CAM binding will probably require a three-dimensional view of all of the extracellular domains that can only come from x-ray crystallographic studies. In the meantime, additional insight may be gained by NMR or x-ray crystallographic analysis of the individual and pairs of the recombinant domains described here.

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