DEVELOPMENTAL BIOLOGY 136, 87-96 (1989)

Latex Beads as Probes of Cell Surface–Extracellular Matrix Interactions during Chondrogenesis: Evidence for a Role for Amino-Terminal Heparin-Binding Domain of Fibronectin

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Accepted June 27, 1989

Fibronectin-rich mesenchymal condensations form at sites of incipient chondrogenesis in the developing vertebrate limb, and in cultures of limb bud mesenchyme. We have used 6 μm polystyrene latex beads coated with various substances as probes for adhesive interactions that may mediate the formation of these condensations. Beads coated with heparin, chondroitin sulfate, or poly L-lysine, that were mixed with limb bud mesenchymal cells were centripetally conveyed into fibronectin-rich regions of cell condensation over a period of several days. Beads coated with dextran sulfate remained uniformly dispersed throughout the cultures during the same period. A monoclonal antibody directed against the amino-terminal heparin-binding domain of fibronectin completely inhibited accumulation of heparin-coated beads at condensing foci, but monoclonal antibodies directed against the collagen- or cell-binding domains of fibronectin were not inhibitory. Accumulation of chondroitin sulfate- or poly L-lysine-coated beads at condensing foci was unaffected by the antibody against the fibronectin amino terminus. Peptides with the sequence arg-gly-asp-ser or gly-arg-gly-asp-ser, which inhibit adhesive interactions mediated by the integrin-binding domain of fibronectin, had no effect on conveyance or accumulation of heparin-coated beads, but the peptide with the sequence gly-arg-gly, a repeated motif in the amino-terminal heparin-binding domain was completely inhibitory. These findings indicate that the amino-terminal heparin-binding domain of fibronectin can, within a tissue microenvironment, interact adhesively with heparin-like components on the surfaces of polystyrene beads, and by implication, on mesenchymal cells themselves. This interaction may therefore be a component of the condensation-forming mechanism in chondrogenic mesenchyme.

INTRODUCTION

Several critical events during vertebrate embryogenesis require the translocation of cells from one site within the embryo to another. Long distance cell translocation occurs during developmental processes such as gastrulation (Critchley *et al.*, 1979), primordial germ cell migration (Critchley *et al.*, 1979), and neural crest cell localization (Le Douarin, 1982; Noden, 1975). Cell movement over relatively shorter distances takes place during cell condensation events, such as those that lead to the formation of cartilaginous bone primordia (Fell and Canti, 1934; Olson and Low, 1971; Thorogood and Hinchliffe, 1975).

During the development of the embryonic chick limb, cell condensations form in a proximodistal sequence, presaging the development of the cartilagenous blastema (Fell and Canti, 1934; Searls *et al.*, 1972; Thorogood and Hinchliffe, 1975; Newman, 1988). Ultrastructurally, in regions of mesenchyme where cartilage will develop, there is an increase in cell packing and an alteration in the specific types of cell-cell interactions (Thorogood and Hinchliffe, 1975). Whereas early limb mesenchyme is characterized by extensive intercellular spaces, after condensation has occurred cells are intimately associated and make broad surface contact with one another (Searls *et al.*, 1972; Thorogood and Hinchliffe, 1975).

Attempts to account for the increase in cell density in precartilage condensations by local increases in mitotic rate have been unsuccessful (Hornbruch and Wolpert, 1970; Janners and Searls, 1970). Some form of cell translocation is thus required to explain the condensation process. While such movements cannot be observed directly in the developing limb, precartilage condensations similar in appearance to those which occur *in situ* occur in cultures of limb mesenchyme (Ede, 1983; Newman, 1977; Solursh *et al.*, 1978). Studies of possible roles for active cell migration (Ede *et al.*, 1977; Solursh, 1984) or of loss of extracellular materials (Toole *et al.*, 1972; Finch *et al.*, 1978) in generating condensations *in vitro* have been inconclusive. Thus, despite numerous previous investigations, there is no well-accepted explanation for the process by which prechondrogenic cells move closer together to establish sites of mesenchymal cell condensation.

Cell translocation events, however, are often mediated by interactions of cells with their extracellular matrices. The macromolecules present within the ex-

0012-1606/89 $3.00

tracellular milieu such as the collagens, fibronectin, hyaluronic acid, and proteoglycans, play an active role in regulating cell behavior by influencing the biosynthetic activities of cells, their shapes, and motility during interactions with one another and with neighboring cell types (Hay, 1981). It has been suggested that local accumulations of an extracellular matrix molecule that encourages cell adhesion, such as fibronectin (Mosher, 1988), could initiate the formation of chondrogenic foci among competent cells (Newman and Frisch, 1979; Newman *et al.*, 1981a). Studies of the distribution of fibronectin during limb development have demonstrated that regions of precartilage cell condensation are indeed rich in this extracellular glycoprotein (Dessau *et al.*, 1980; Tomasek *et al.*, 1982).

Earlier work has shown that adhesive interactions with the embryonic microenvironment are capable of conveying latex beads along specific pathways during early embryogenesis (Bronner-Fraser, 1982). Our previous studies on the behavior of such beads, and limb mesenchymal cells, in artificial collagen-fibronectin matrices, demonstrated an interaction between the cell or bead surface and the amino-terminal domain of fibronectin that was capable of exerting a translocational force (Newman *et al.*, 1985, 1987). This effect, termed "matrix-driven translocation," appears to depend on a conformational change in fibronectin upon its binding to heparin-like ligands on the bead or cell surface (Khan *et al.*, 1988).

These previous studies suggested the possibility of using coated latex beads as probes of the extracellular adhesive environment during the condensation phase of chondrogenesis. Unlike mesenchymal cells themselves, which present a complex cell surface to a comparably complex matrix, latex beads can be coated uniformly with a homogenous macromolecule. Interactions and associations with matrix components that lead to changes in bead distribution can then be studied by specifically blocking matrix components, while holding the surface composition of the beads constant. Because the latex beads are nondeformable as well as being intrinsically nonmotile, this technique can also be used to determine the extent to which adhesive forces generated at the cell surface-extracellular matrix interface can potentially cause passive rearrangement of mesenchymal cells themselves.

We have found that a bead surface-extracellular matrix interaction with the same fibronectin domain specificity as matrix-driven translocation promotes the translocation of heparin-coated beads into regions of high fibronectin concentration in cultures of limb bud mesenchyme cells. Beads coated with chondroitin sulfate A or B, or poly L-lysine, are conveyed into such regions by surface-matrix interactions with different specificities. In contrast, beads coated with dextran sulfate remain dispersed throughout the cell mass. We suggest that precartilage mesenchymal cells themselves may be drawn into tissue regions rich in certain extracellular matrix components by means of adhesive forces similar to these which act on the beads, giving rise to the characteristic condensations seen in culture and in the developing limb.

MATERIALS AND METHODS

Cell culture. Chick limb precartilage mesenchymal cells were prepared from the distal tip of the wing buds of stage 25 (Hamburger and Hamilton, 1951) White Leghorn chick embryos, as previously described (Newman, 1977, 1980). In some experiments, a mixed population of premuscle and precartilage mesenchymal cells was prepared from whole wing buds of stage 22/23 embryos (Newman *et al.*, 1981b). Cells were cultured using the micromass technique (Ahrens *et al.*, 1977). Briefly, following trypsinization, cells were resuspended in Ham's F-12 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) at a density of 2.5×10^7 cells per ml. Ten-microliter droplets of the cell suspension, containing 2.5×10^5 cells, were placed in the centers of wells in a 24 well tissue culture plate (Falcon 3047, or Costar 3424). After 1 hr of incubation at 37°C, 1 ml of Ham's F-12 media supplemented with 10% FBS was added to each well.

In most experiments coated latex beads were added to the cultures. Six μm polystyrene latex beads (Polysciences 7312), sterilized in 70% ethanol and washed extensively with saline solution prior to coating as described below, were counted in a hemocytometer and mixed with the mesenchymal cells in the ratio of 5% beads to 95% mesenchymal cells.

In some experiments, monoclonal antibodies directed against specific fibronectin domains (10–30 μg/ml) or synthetic peptides (10–100 μg/ml) were added to the culture medium. Media, with appropriate additives, were completely changed each day. Selected microscopic fields were photographed with phase contrast and bright field optics on an Olympus inverted microscope.

Preparation of coated latex beads. Six micrometer polystyrene latex beads were coated with defined macromolecules, as previously described (Newman *et al.*, 1987). Briefly, the beads were incubated in 0.5 mg per ml poly-L-lysine (Sigma, P-1399) for 30 min at room temperature. These were used as such, or further coated with heparin (a gift from Dr. I. Danishefsky), chondroitin sulfate type A (Sigma C-8529) or type B (Sigma C-4259), or dextran sulfate (Sigma D-6393 or D-8906), by incubation for 30 min at room temperature in 12.5 mg/per ml of these substances. Binding of the glycos-

aminoglycans was confirmed using the carbazole method (Bitter and Muir, 1962) and was typically 200–300 μg per 1×10^7 beads.

Monoclonal antibodies and peptides. Antibodies directed against the 31-kDa amino-terminal heparin-binding domain, the 43-kDa collagen-binding domain, and the 75-kDa cell receptor binding domain of fibronectin (Newman *et al.*, 1987) were added to cultures as intact immunoglobulins purified from conditioned, serum-free medium. Fab fragments of antibody 304, were prepared by digestion of the antibody with immobilized papain (Pierce Chemical Co., Rockford, IL) for 6 hr at 37°C, and purified according to the manufacturer's instructions. Peptides were synthesized by Peninsula Laboratories Inc. (Belmont, CA) and purified by reverse-phase high-performance liquid chromatography.

Immunofluorescent staining for fibronectin. Micromass cultures were grown on glass coverslips in Ham's F-12 medium containing 10% FBS from which endogenous fibronectin had been removed by two passes through a gelatin-sepharose affinity column (Engvall *et al.*, 1978). Cultures were fixed in 4% paraformaldehyde in 0.1 *M* phosphate buffer pH 7.2 for 10 min at 4°C, washed three times for 5 min with phosphate buffered saline (PBS) and three times for 5 min with 0.1 *M* Tris buffer pH 7.2. Goat anti-fibronectin antibody (a generous gift from Dr. Hynda Kleinman) was applied for 30 min at room temperature. After washing the cells free of primary antibody, rabbit anti-goat IgG (Cappell) was applied for 30 min at room temperature. Cells were washed free of secondary antibody and coverslips were mounted in glycerol/PBS (9:1). Cells were examined with a Leitz Dialux epifluorescence microscope.

RESULTS

Translocation of Latex Beads into Mesenchymal Condensations

Polystyrene latex beads coated with defined macromolecules were mixed with chick limb bud precartilage cells prepared from stage 25 wing tips, in a 5:95 ratio, and the mixtures were plated as micromass cultures. In cultures prepared from these uniformly prechondrogenic cells, a small number (<10) of large foci of condensation are typically apparent by the second day after plating (Gay and Kosher, 1984). The addition of polystyrene latex beads with any of the coatings used had no effect on the number of condensations formed.

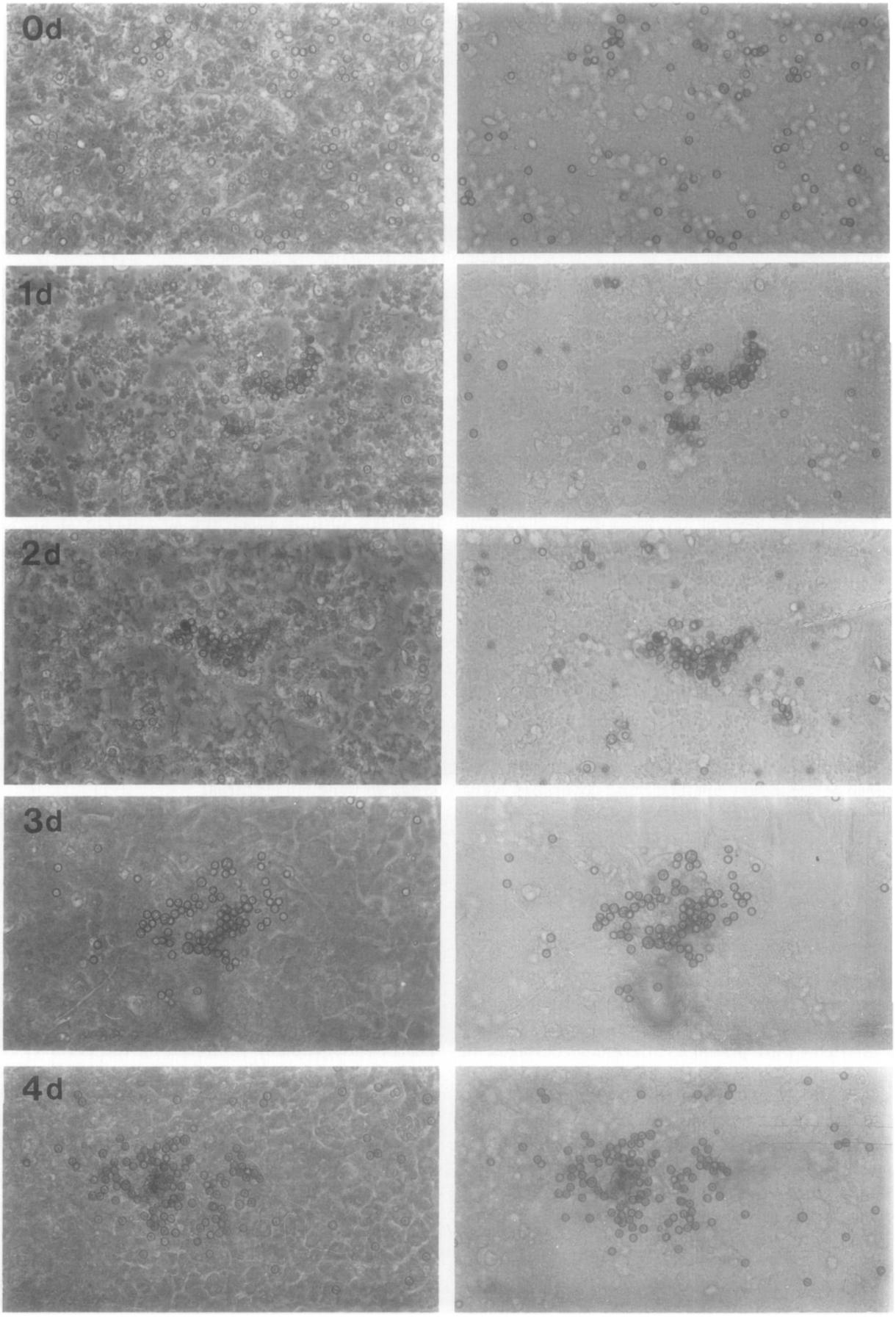
Latex beads that contained a surface coat of heparin were dispersed throughout the cell mass on the initial day (Day 0) of culture (Fig. 1). On Day 1, some heparin-coated beads had been conveyed into sites that by the following day were always unambiguous foci of condensation. We marked the sites of these initial bead aggregations and followed them over the subsequent several days. By Days 2 and 3 in culture, heparin-coated beads had been translocated over distances of several cell diameters into definitive sites of precartilage mesenchymal cell condensations. This was in contrast to the cells themselves, whose average centripetal translocation during this period was less than one cell diameter (Ede *et al.*, 1977). By Day 4, large numbers of beads throughout the depth of the cultures had been conveyed into regions of mesenchymal cell condensation and had accumulated at these sites. Beads that had been coated with chondroitin sulfate A or B, or poly L-lysine were similarly conveyed into foci of mesenchymal condensation over the same period (not shown). In contrast, beads that had been coated with dextran sulfate remained dispersed throughout the tissue mass during the entire culture period, even at sites of mesenchymal cell condensation (Fig. 2).

Similar results were obtained in cultures of stage 22/23 whole limb buds. When cultured alone, or with beads, these cells typically form more than 50 small foci of condensation by the second day after plating. Heparin-, chondroitin sulfate- or poly L-lysine-coated beads mixed with these cells were conveyed into these small condensations in a similar fashion to the movement of beads into the larger condensations of the stage 25 precartilage cell cultures. In stage 22/23 cultures 10 or more of the condensations generally contained between 5 and 15 beads after 4 days in contrast to the 50 or more beads typically found in the larger condensations of the stage 25 wing tip cultures.

We examined the relationship between fibronectin distribution in these cultures and the accumulation of heparin-coated beads by immunostaining using an antifibronectin antibody. High density cultures containing heparin-coated beads were prepared with either stage 25 limb tip mesenchyme, or stage 22/23 whole limb mesenchyme and grown in medium free of serum fibronectin. As early as Day 1 in culture, before condensations were evident, clusters of heparin-coated beads were observed to codistribute with cellular fibronectin endogenously produced by the cultures (Fig. 3). Definitive condensations were present on the following day (Frenz, *et al.*, 1989). These were foci of both bead accumulation (Fig. 1) and of fibronectin deposition. These sites stained for fibronectin in a graded fashion from center to periphery (Fig. 4).

Effect of Antibodies Directed Against Specific Fibronectin Domains on Bead Translocation in Culture

To determine whether translocation of heparin-coated beads in chondrogenic cultures depended on in-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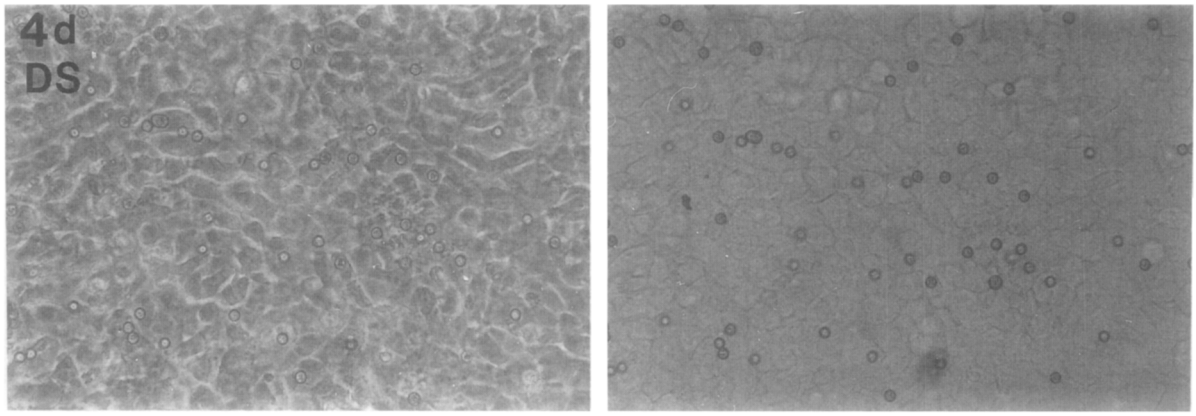

FIG. 2. Lack of translocation of dextran sulfate-coated beads into precartilage cell condensations. Cultures containing mixtures of stage 25 wing tip mesenchyme and dextran sulfate-coated beads (95:5) were prepared and monitored by phase contrast (left panel) and bright field (right panel) microscopy over a 4-day culture period. On day 4, dextran sulfate-coated beads remained dispersed throughout the tissue mass.

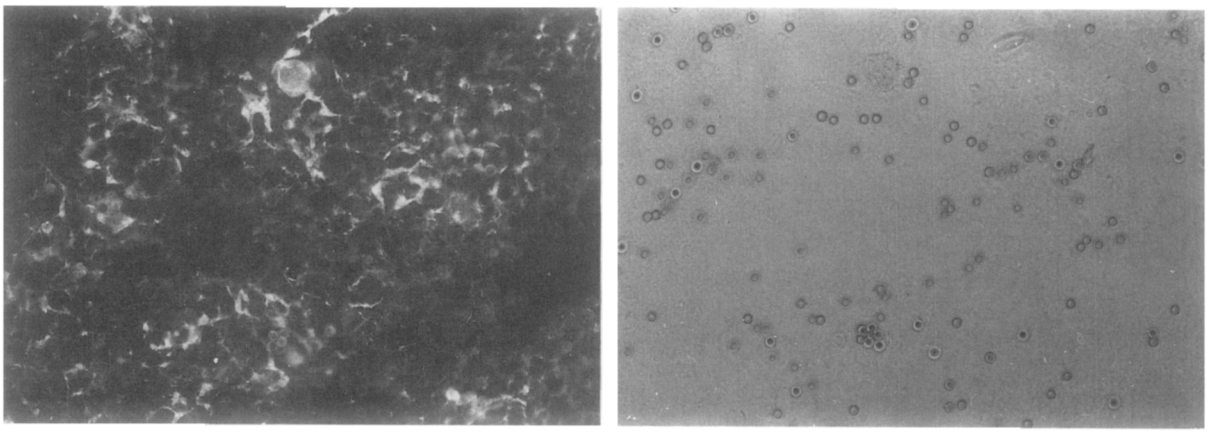

FIG. 3. Relationship of the distribution of heparin-coated beads to fibronectin in Day 1 precartilage cell cultures. High density cultures were prepared containing mesenchymal cells isolated from whole stage 22/23 wing buds and heparin-coated beads (95:5). Medium contained fetal bovine serum from which plasma fibronectin had been removed. (Left panel) Indirect immunofluorescence micrograph of a Day 1 culture showing the distribution of endogenous cellular fibronectin. (Right panel) Bright field micrograph of the same microscope field showing the localization of heparin-coated beads.

teractions with fibronectin, we cultured stage 25 wing tip precartilage mesenchymal cells, or stage 22/23 whole wing mesenchymal cells, along with coated beads, in the presence of monoclonal antibodies directed against various domains of the glycoprotein. Results for stage 25 cultures are shown in Fig. 5. Results for the 22/23 cultures were virtually identical. In the presence of monoclonal antibodies 191 and 333 (30 μg/ml), directed against the collagen-binding or cell receptor-binding domains of fibronectin, respectively, translocation and aggregation of heparin-coated beads occurred at sites of mesenchymal condensation exactly as if no monoclonal antibodies were present (Fig. 5). However, in the presence of monoclonal antibody 304 (10 μg/ml), directed against the amino-terminal heparin-binding domain of fibronectin, there was a complete inhibition of translocation and accumulation of heparin-coated beads (Fig. 5). Fab fragments prepared from antibody 304 (10 μg/ml) were as effective as intact antibody in preventing translocation of heparin-coated beads into

FIG. 1. Translocation of heparin-coated beads into precartilage cell condensations. Cultures contained mixtures of stage 25 wing tip mesenchymal cells and heparin-coated beads (95:5). Cultures were monitored by phase contrast microscopy (left panel) and bright field microscopy (right panel). Because of the thickness of the micromass cultures, many cells and beads are out of the plane of focus. Each phase contrast-bright field pair corresponds to the same microscopic field. On the initial day of culture (0d), heparin-coated beads were dispersed throughout the cell mass. In the series denoted by 1d, 2d, 3d, and 4d, a single site of incipient condensation is shown over a period of 4 days. Beads are 6 μm in diameter.

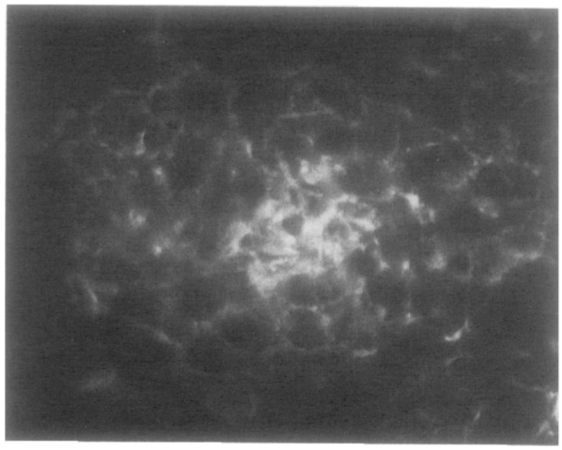

FIG. 4. Distribution of endogenously produced fibronectin in Day 2 mesenchymal cultures. Cultures were prepared in fibronectin-free medium and stained for fibronectin on the 2nd day after plating. Fibronectin stained in a graded fashion in each focus of condensations, with the maximal staining at the center.

mesenchymal condensations indicating that the inhibitory effect was the result of the blocking of a specific function of fibronectin, rather than nonspecific cross-linking of this molecule. While the concentrations of antibody 304 used here reduced the number of condensations by up to 66% (Frenz *et al.*, 1989), beads coated with chondroitin sulfate or poly L-lysine accumulated at those sites of condensation that continued to form in the presence of the antibody. A summary of the effects of antibody 304 on the accumulation of beads with various coatings is given in Table 1.

Effect of Fibronectin-Related Peptides on Bead Translocation in Culture

The preceding experiments did not exclude the possibility that the cell binding or the collagen-binding domains of fibronectin were important in the accumulation of heparin-coated beads at the sites of condensation but that these domains were inaccessible to the corresponding antibodies *in situ*. To further explore the domain specificity of bead translocation, we cultured mixtures of mesenchymal cells and heparin-coated beads in the presence of peptides related to amino acid sequences in different fibronectin domains. In the presence of the synthetic peptide with the sequence arg-gly-asp-ser (RGDS[1]; 10-100 μg/ml) or gly-arg-gly-asp-ser (GRGDS; 10-100 μg/ml) which are competitive inhibitors of the binding site of fibronectin for the integrin family of cell surface receptors (Hynes, 1987; Ruoslahti and Pierschbacher, 1987), heparin-coated beads were translocated into, and accumulated at precartilaginous foci like they were in control experiments in which no synthetic peptides were added. In contrast, in the presence of comparable amounts of the tripeptide with the sequence gly-arg-gly (GRG), a repeated motif in both the amino-terminal heparin-binding domain and the collagen-binding domain of fibronectin (Kornblihtt *et al.*, 1985), or the related tetrapeptide gly-arg-gly-asp (GRGD), which does not interfere with RGDS-dependent functions (Yamada and Kennedy, 1987), a marked inhibition of translocation of heparin-coated beads into precartilaginous condensations was observed. This inhibition became complete at higher peptide concentrations. Rather than localizing within foci, the beads remained dispersed throughout the surrounding tissue mass (Table 2). There was no evident change in the overall amount or pattern of fibronectin immunofluorescence in cultures treated with either inhibitory peptide (not shown).

[1] Abbreviations used: GRG, gly-arg-gly; GRGD, gly-arg-gly-asp; GRGDS, gly-arg-gly-asp-ser; RGDS, arg-gly-asp-ser.

DISCUSSION

The rearrangement of cells during embryonic morphogenesis, cancer cell invasion, inflammation, and other normal and abnormal tissue remodeling processes depends critically on adhesive interactions between cells and their extracellular matrices. But whereas "adhesion" comprises a wide variety of physical and chemical effects, the biological importance of any such interaction arises from the possibility of its occurrence among cells situated in complex tissue environments. Cell surface receptors and extracellular matrix macromolecules that may exhibit affinities for one another on planar substrata, for example, may not necessarily be

TABLE 1

EFFECT OF ANTI-FIBRONECTIN AMINO-TERMINUS MONOCLONAL ANTIBODY ON ACCUMULATION OF COATED BEADS AT CONDENSATION SITES

Bead coating	Culture conditions	
	−Mab304	+Mab304[a]
Poly-L-lysine	+[b]	+
Chondroitin sulfate A	+	+
Chondroitin sulfate B	+	+
Heparin	+	−

[a] Antibody was present at 10 μg/ml for duration of culture period.

[b] Results were scored after 3 days of culture; stage 22/23 wing mesenchyme was used. A score of + indicates the presence at least 10 condensations (5 in the presence of the antibody) with more than six beads in each; a score of − indicates that no site in the culture contained more than three contiguous beads. Each bead type was assayed in at least three independent experiments, which all gave identical results within the stated criteria.

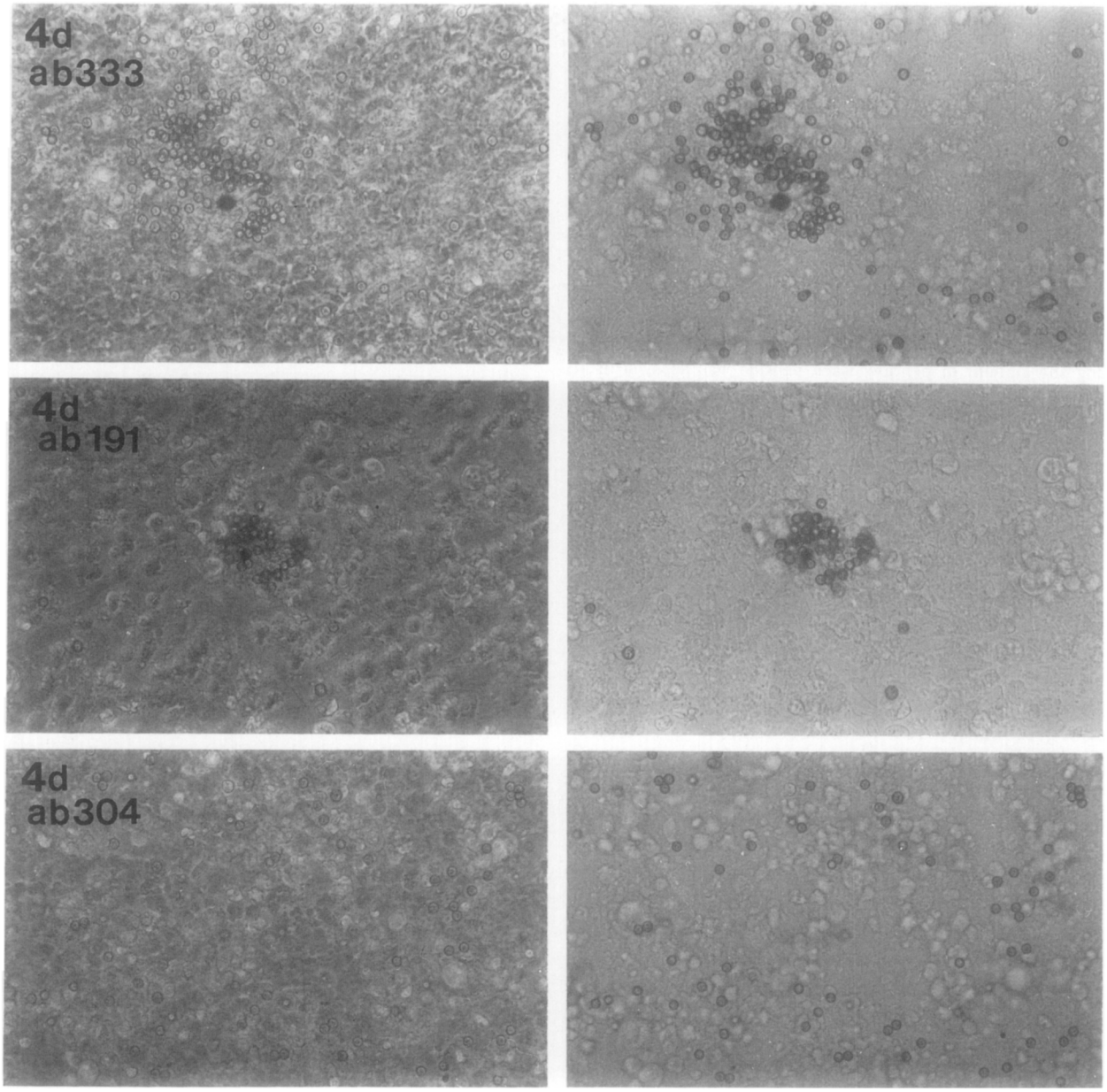

FIG. 5. Effect of antifibronectin monoclonal antibodies on bead translocation into precartilage condensations. Cultures containing mixtures of stage 25 wing tip mesenchymal cells and heparin-coated beads were grown in the presence of monoclonal antibodies (30 μg/ml) directed against the collagen-binding domain (Mab 191), the cell-binding domain (Mab 333), and the amino-terminal heparin-binding domain of fibronectin (Mab 304). Cultures were examined for the presence of aggregates of beads over 4 days. Only in the presence of Mab 304 was bead translocation into regions of cell condensation prevented. Similar results were obtained using 10 μg/ml Mab 304 or its Fab fragment.

accessible for interaction when apposed to one another in an extracellular matrix. Moreover, whether a particular adhesive interaction will promote cell immobilization or cell movement depends critically on the balance of forces at the cell–matrix interface during tissue morphogenesis (Forgacs *et al.*, in press).

By introducing cell-sized test particles coated with a relatively homogeneous single type of ligand (e.g., heparin) into mesenchymal cultures undergoing morphogenesis, we have attempted to simplify the analysis of adhesive interactions during development. The possibility of cellular translocation resulting from interactions at the cell surface–extracellular matrix interface may be assayed in this system under conditions in which relative adhesive affinities play a decisive role, and chemokinesis and chemotaxis are not relevant.

Adhesive forces alone, which are directed normal to the cell–cell or cell–matrix interface are not sufficient

TABLE 2
EFFECT OF SYNTHETIC PEPTIDES ON ACCUMULATION OF HEPARIN-COATED BEADS AT CONDENSATION SITES

Peptide		Translocation
RGDS	(10–100 μg/ml)	+[a]
GRGDS	(10–100 μg/ml)	+
GRGD	(10 μg/ml)	±
	(15–100 μg/ml)	−
GRG	(50 μg/ml)	±
	(100 μg/ml)	−

[a] Results were scored as in Table 1. A score of ± represented intermediate results, but in all such cases a score of − could be achieved by increasing the amount of peptide. Each peptide was assayed in at least three independent experiments; in a given experiment each treatment group comprised three separate cultures.

to account for translocation tangential to these surfaces. What is required for translocation is a means by which cells or inert particles can randomly sample their local microenvironments and establish adhesive interactions that are more energetically favorable than those at their original sites. The intrinsic random motility of cells provides for this sampling in heterotypic mixtures of cells undergoing sorting-out behavior (Steinberg, 1962a,b; 1970; Armstrong, 1989). Analogously, in the cell-bead mixtures we have studied, the motility of the surrounding mesenchymal cells appears sufficient to expose the nonmotile particles to different microenvironments.

Under these conditions, inert particles, or cells themselves, have the potential to move up adhesive gradients of extracellular macromolecules, providing the following requirements are met: (i) the potentially adhesive cell or particle surface and extracellular matrix components have access to one another in the presence of other tissue components; (ii) the relative concentrations of interacting components are such that successively stronger adhesive interactions can be established over macroscopic distances in the tissue, and (iii) adhesive "bonds" can be readily made and broken, so that the formal potential to generate stronger interactions by positional change can be realized in practice.

In our cell-bead mixtures all three of the above requirements appear to be met by the interaction between beads coated with heparin, chondroitin sulfate, or polylysine, and the adhesive environment present in developing precartilage mesenchyme cells. Such beads accumulated at foci of condensation, in contrast to beads coated with dextran sulfate, which remained scattered throughout the cell mass.

It seems likely that the accumulation of heparin-coated beads at foci of mesenchymal condensation was mediated in part by the graded distribution of fibronectin established around each incipient focus at 1–2 days. Not only did the beads colocalize with fibronectin at the light microscopic level from the start of the accumulation process, the accumulation could be completely inhibited by an anti-fibronectin monoclonal antibody or a peptide (GRG) representing a repeated motif in fibronectin.

The ineffectiveness of antibodies 191 and 333, and the peptide RGDS, in preventing translocation of heparin-coded beads, together with the lack of obvious ligands on the beads for the collagen or integrin binding domains of fibronectin argue against the involvement of these domains in the interaction studied here. But since none of our reagents was specifically directed to the heparin binding domain in the carboxy-terminal region of fibronectin (Hayashi *et al.*, 1980; Hayashi and Yamada, 1982), we cannot exclude a role for this site, or any other heparin binding component that may be present in the extracellular matrix, in mediating directional translocation of heparin-coated beads into condensing foci in the cultures. We note, however, that inhibition of translocation by antibody 304 shows that the amino-terminal heparin binding domain of fibronectin is a necessary component of this process.

Because GRG, which is present twice in the amino-terminal domain of fibronectin (Kornblihtt *et al.*, 1985), stopped the accumulation of heparin-coated beads, the peptide results also support a role for this domain in the interaction. Indeed, we have found that GRG specifically inhibits binding of the fibronectin amino terminal domain to heparin (M. Y. Khan and S. A. Newman, unpublished results).

While the accumulation of heparin-coated beads at sites of mesenchymal condensation appears, therefore, to be mediated in part by adhesion to a well-characterized domain of fibronectin, accumulation of chondroitin sulfate-coated or polylysine-coated beads was not inhibited by the antibody directed against the fibronectin amino-terminus. Chondroitin sulfate-coated beads may be conveyed into regions of mesenchymal condensation by interaction with other domains of fibronectin, or with other matrix components that are concentrated in these centers, while accumulation of beads coated with polylysine probably depends on one or more of the diverse adhesive interactions of which this polycation is capable. The failure of dextran sulfate-coated beads to accumulate at condensation foci demonstrates that neither charge nor exposed sulfate groups on a surface-bound polysaccharide are sufficient to mediate the interaction required for translocation. Our earlier finding that heparin induces a conformational change in the amino-terminal domain of fibronectin which is different from that induced by dextran sulfate (Khan *et al.*, 1988) may be significant in this regard.

Chick limb precartilage mesenchymal cells contain a surface coat of heparin-like molecules whose removal makes these cells unsusceptible to an adhesive interaction with the amino-terminal domain of fibronectin (Newman *et al.*, 1987). These surface components probably consist of heparan sulfate proteoglycan (Vasan, 1986). Precartilage cells, like heparin-coated beads, thus have the potential to interact adhesively with the amino-terminal domain of fibronectin in the extracellular microenvironment. If fibronectin accumulates nonuniformly within the limb mesenchyme, as it appears to both *in situ* (Dessau *et al.*, 1980; Tomasek *et al.*, 1982) and in culture (Fig. 4), it is reasonable to infer that cells will be drawn centripetally into the fibronectin-rich foci by interactions similar to those leading to accumulation of beads at these sites.

Studies reported in the accompanying paper (D. Frenz, N. S. Jaikaria, and S. A. Newman, 1989. *Dev. Biol.* **135,** 97–103) show that monoclonal antibody 304, GRG and GRGD, or *Flavobacterium* heparinase, can reduce the number of mesenchymal condensations, to less than 50% of control values (Frenz, *et al.*, 1989). While this suggests that the fibronectin-dependent interaction probed with heparin-coated beads is a major component of the condensation-forming mechanism, it leaves open the possibility that other cell surface-extracellular interactions, possibly dependent on cell surface chondroitin sulfate, may also play important roles in this process.

We thank N. S. Jaikaria for suggesting the studies with GRG-containing peptides. This work was supported by a grant from the National Science Foundation to S.A.N. (DCB-8609106), from the National Institutes of Health to S.A.N. (HD22564), S.K.A. (CA45515), Howard University (CA14718), and the Morehouse School of Medicine (RR08248), and from the American Cancer Society to Dr. Kenneth Olden. S.K.A. thanks Dr. Kenneth Yamada for advice and encouragement.

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