Human Fibroblast Adhesion to Fibrinogen[†]

David H. Farrell* and Hamid A. Al-Mondhiry[‡]

Department of Biochemistry and Molecular Biology, and Department of Medicine, The Pennsylvania State University, College of Medicine, P.O. Box 850, Hershey, Pennsylvania 17033

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ABSTRACT: Fibrinogen and fibrin mediate the adhesion of many cell types. In this report, the adhesion sites for human dermal fibroblasts on fibrinogen are identified and characterized. Fibroblasts showed a time- and dose-dependent adhesion to fibrinogen. Using a combination of synthetic peptide mimetics, monoclonal antibodies, and recombinant fibrinogens, two major classes of adhesive sites were identified. One class was RGD-dependent and involved the RGD sites in the α chain of fibrinogen. α_V integrins present on fibroblasts appeared to mediate this adhesion. Inhibition studies showed that the RGD-independent site was blocked by an ICAM-1 antagonist peptide. Furthermore, the inhibition was additive with RGD peptide inhibition and accounted for essentially all of the fibroblast adhesion. Together, these results suggest that fibroblast adhesion to fibrinogen is mediated by both α_V integrins and ICAM-1.

Fibrinogen and fibrin contain multiple adhesion sites for several different cell types. Platelets (Bennett & Vilaire, 1979; Marguerie et al., 1979), monocytes (Gonda & Shainoff, 1982), leukocytes (Altieri et al., 1986), neutrophils (Wright et al., 1988), vascular endothelial cells (Kadish et al., 1979; Dejana et al., 1985), smooth muscle cells (Naito et al., 1992), keratinocytes (Donaldson & Mahan, 1983), and fibroblasts (Colvin et al., 1979; Dejana et al., 1984) all contain cell surface adhesive receptors for fibrin(ogen). Many studies have implicated these adhesive interactions in key physiological roles such as platelet aggregation, angiogenesis, leukocyte−endothelium adhesion, and wound healing. Most of the known fibrinogen receptors are integrins, although one receptor is a member of the immunoglobulin superfamily (Languino et al., 1993), intercellular adhesion molecule 1 (ICAM-1). The integrins include the platelet receptor $\alpha_{IIb}\beta_3$ (glycoprotein IIb-IIIa) (Phillips & Agin, 1977), the vitronectin receptor $\alpha_V\beta_3$ (Fitzgerald et al., 1985), the leukocyte integrin $\alpha_M\beta_2$ (CD11b/CD18; Mac-1; CR3) (Altieri et al., 1988; Wright et al., 1988), and the neutrophil integrin $\alpha_X\beta_2$ (CD11c/CD18; p150/95) (Loike et al., 1991; Postigo et al., 1991).

Fibrinogen is a dimer of three chains, the α, *β*, and *γ* chains. The cell receptor binding sites on fibrinogen have been mapped to regions of the α and *γ* chains using blocking antibodies, peptidyl mimicry, and mutational analysis. A dodecapeptide sequence at the carboxy terminus of the *γ* chain from *γ*400−411 mediates fibrinogen binding to platelets via $\alpha_{IIb}\beta_3$ (Kloczewiak et al., 1982, 1983, 1984). A different sequence in the *γ* chain from amino acids *γ*117−133 mediates fibrinogen bridging of leukocytes and endothelium via ICAM-1 (Altieri et al., 1995). Yet another *γ* chain sequence from *γ*190−202 mediates fibrinogen binding to leukocytes via $\alpha_M\beta_2$ (Altieri et al., 1993), while an α chain sequence from α17−19 mediates fibrinogen binding to neutrophils via $\alpha_X\beta_2$ (Loike et al., 1991). Fibrinogen also contains two canonical Arg-Gly-Asp (RGD) sites in the α chain at positions α95−97 and α572−574. The role of these sites in cell adhesion has been a subject of controversy. RGD-containing peptides bind to platelet $\alpha_{IIb}\beta_3$ and inhibit fibrinogen-mediated platelet aggregation (Gartner & Bennett, 1985; Haverstick et al., 1985; Plow et al., 1985). Furthermore, proteolytic fragments of fibrinogen that lack the RGD sites have decreased platelet adhesion (Savage et al., 1995). However, mutation of these sites to the inactive RGE sequence has no effect on platelet aggregation or adhesion (Farrell et al., 1992; Farrell & Thiagarajan, 1994), even under flow conditions (Zaidi et al., 1996). This suggests that the RGD sites in native fibrinogen may not be recognized by $\alpha_{IIb}\beta_3$. In contrast, several studies have shown that endothelial cell $\alpha_V\beta_3$ binds to fibrinogen via the RGD at position α572−574 (Cheresh et al., 1989; Thiagarajan et al., 1996). Thus, different binding sites in fibrin(ogen) support the adhesion of different cell types.

Fibroblast adhesion to fibrinogen plays a significant role in wound healing. The growth of fibroblast arrays leads ultimately to the formation of scar tissue at wound sites (Beck et al., 1961). Fibroblasts migrating into a wound site encounter different types of matrix components, including fibrin(ogen), collagen, and fibronectin. However, there are contradictory reports in the literature regarding fibroblast adhesion to fibrinogen. Studies in the early 1980s showed that fibroblastic cells did not adhere to fibrinogen; any adhesion to fibrinogen in these studies could be accounted for by contaminating fibronectin in the fibrinogen preparations (Grinnell et al., 1980; Pierschbacher & Ruoslahti, 1984a). However, it was not clear whether these results could be generalized to normal diploid human fibroblasts, since the experiments were performed with immortalized rodent cell lines (BHK and NRK cells, respectively) that have different adhesion receptors. Other studies using human

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* To whom correspondence should be addressed, at the Department of Biochemistry and Molecular Biology, The Pennsylvania State University, College of Medicine, P.O. Box 850, Hershey, PA 17033. Tel: (717) 531-4098. FAX: (717) 531-7072. E-mail: dfarrell@bcmic.hmc.psu.edu.

[‡] Department of Medicine, The Pennsylvania State University, College of Medicine, P.O. Box 850, Hershey, PA 17033. Tel: (717) 531-8401. FAX: (717) 531-6094.

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dermal fibroblasts showed that human fibroblasts adhere to fibrin made from highly-purified fibrinogen (Brown et al., 1993), but exhibit a rounded morphology and do not spread on fibrinogen alone (Sporn et al., 1995). Spreading on fibrinogen requires the conversion of fibrinogen to fibrin, specifically the cleavage of fibrinopeptide B (Chalupowicz et al., 1995; Sporn et al., 1995). Migration of fibroblasts on fibrin was greatly enhanced by cross-linking of the fibrin by factor XIII (Beck et al., 1961; Colvin et al., 1979; Brown et al., 1993). Fibroblasts also mediate fibrin clot retraction, similar to platelets (Niewiarowski et al., 1972). This clot retraction process can be inhibited by RGD-containing peptides (Tuan & Grinnell, 1989), suggesting the involvement of integrin-type receptors. In addition, confluent cultures of fibroblasts display receptors for the central E-domain of fibrinogen on their apical surface (Dejana et al., 1984). However, the epitopes on fibrinogen required for the initial adhesion step remain incompletely characterized. In this report, the adhesion sites on fibrinogen for human fibroblasts are characterized. Using peptide mimetics, monoclonal antibodies, and recombinant fibrinogens, we show that the initial adhesion of human dermal fibroblasts to fibrinogen is mediated by both RGD-dependent and independent mechanisms.

EXPERIMENTAL PROCEDURES

Purification of Plasma-Derived Fibrinogen. Human blood was collected from anonymous donors with informed consent in 0.1 volume of 0.055 M trisodium citrate, pH 7.4/0.1 M ϵ-aminocaproic acid/0.1 mM phenylmethanesulfonyl fluoride/1 mM benzamidine. Fibrinogen was isolated by glycine precipitation as described before (Kazal et al., 1963) as modified by Martinez et al. (1974) in the presence of the protease inhibitors. In control experiments, the fibrinogen was passed over a column of gelatin−agarose (Sigma Chemical Co., St. Louis, MO) to remove contaminating fibronectin (Engvall & Ruoslahti, 1977). The purified fibrinogens were analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (Laemmli, 1970).

Production of Recombinant Fibrinogens. Recombinant fibrinogen molecules were designed with mutations in potential cell adhesion domains. The γ chain dodecapeptide was interrupted at position 408 (numbered according to Chung et al., 1983) by substituting the γ' variant chain in rFbgγ',[1] as described previously (Farrell et al., 1991). This results in the synthesis of homodimeric γ'-containing fibrinogen (rFbgγ') with the stoichiometry ($\alpha\beta\gamma'$)($\alpha\beta\gamma'$), rather than the heterodimeric form found in plasma, ($\alpha\beta\gamma$)($\alpha\beta\gamma'$). The RGD sequences at positions α95−97 and α572−574 (numbered according to Doolittle et al., 1979; Henschen et al., 1980) were mutated to RGE as described previously (Farrell et al., 1992) to produce rFbgD97E and rFbgD574E, respectively. The choice for this conservative substitution was based on synthetic peptide binding studies (Pierschbacher & Ruoslahti, 1984a,b) and on the binding of recombinant von Willebrand factor (Beacham et al., 1992), recombinant vitronectin (Cherny et al., 1993), and recombinant fibrinogen (Thiagarajan et al., 1996), in which the RGD to RGE mutation significantly reduced adhesive activity in each protein. The double mutant rFbgD97E/D574E was made by mutating both RGD sequences at positions α95−97 and α572−574 to RGE. Recombinant fibrinogens were purified from the serum-free medium of transfected baby hamster kidney cells as described in detail previously (Farrell & Thiagarajan, 1994).

Cell Culture. Human neonatal foreskin fibroblasts (generously provided by Dr. H. Paul Ehrlich, Penn State College of Medicine, Hershey, PA) were grown in Dulbecco's modified Eagle medium/10% fetal bovine serum/100 units/mL penicillin/100 μg/mL streptomycin (Gibco BRL, Gaithersburg, MD). The human microvascular endothelial cell line HMEC-1 (generously provided by Drs. Edwin W. Ades and Thomas J. Lawley, Centers for Disease Control, Atlanta, GA) was grown in MCDB 131/10% fetal bovine serum/10 ng/mL epidermal growth factor (Collaborative Biomedical Products, Bedford, MA)/1 μg/mL hydrocortisone (Sigma Chemical Co.)/100 units/mL penicillin/100 μg/mL streptomycin (Ades et al., 1992). All cells were grown at 37 °C in a 5% CO_2 atmosphere.

Cell Adhesion Assays. Cell adhesion assays were performed as described previously (Haverstick et al., 1985). Briefly, 200 μL of 0.15 M NaCl/20 mM Tris, pH 7.4, containing various amounts of fibrinogen was added to 96 well Immunoplates (Nunc, Inc., Naperville, IL) and incubated overnight at 4 °C. The solutions were aspirated, and the wells were blocked with 3% bovine serum albumin/0.15 M NaCl/20 mM Tris, pH 7.4 (Sigma Chemical Co.). Confluent monolayers of cells were detached by incubation in 0.25% trypsin/1 mM EDTA (Gibco BRL) for 5 min at 37 °C. The trypsin and EDTA were neutralized by the addition of the appropriate tissue culture medium containing 10% fetal bovine serum. The cells were centrifuged and resuspended in 500 μL of serum-free medium with 100 μCi of $Na^{51}CrO_4$ (DuPont NEN, Wilmington, DE) for 1 h at 37 °C. The cells were washed three times in serum-free medium and resuspended at 10^6 cells/mL. Aliquots of 100 μL were added to fibrinogen-coated wells and incubated for various times from 0.5 to 4 h at 37 °C. In certain experiments, the cells were incubated in the presence of 500 μM synthetic peptides GRGDSP or GRGESP (Gibco BRL), the peptide HHLGGAKQAGDV (Peninsula Laboratories, Belmont, CA), or the γ3 peptide NNQKIVNLKEKVAQLEA (generously provided by Dr. Dario Altieri, Yale University, New Haven, CT). In other experiments, the cells were incubated in the presence of blocking monoclonal antibodies directed against integrin α_V (MAB 1980, Chemicon International, Inc., Temecula, CA), integrin α_M (OKM1, generously provided by Dr. Edward Plow, Cleveland Clinic Foundation, OH), or integrin β_2 (also provided by Dr. Plow). After incubation, nonadherent cells were removed by washing three times in 200 μL of serum-free medium. Microscopic examination of the wells showed similar amounts of cells bound to wild-type fibrinogen controls in all cases. The extent of adhesion was determined by solubilizing the cells with two portions of 200 μL of 2% sodium dodecyl sulfate for 20 min each, combining the extracts, and counting the ^{51}Cr in a γ-counter. Background adhesion to bovine serum albumin-coated wells was subtracted from each data point.

[1] Abbreviations: Fbg, plasma fibrinogen; rFbg, recombinant fibrinogen; rFbgD97E, recombinant fibrinogen containing a D97E mutation in the α chains; rFbgD574E, recombinant fibrinogen containing a D574E mutation in the α chains; rFbgD97E/D574E, recombinant fibrinogen containing a D97E and D574E mutation in the α chains; rFbgγ', recombinant fibrinogen containing γ' chains.

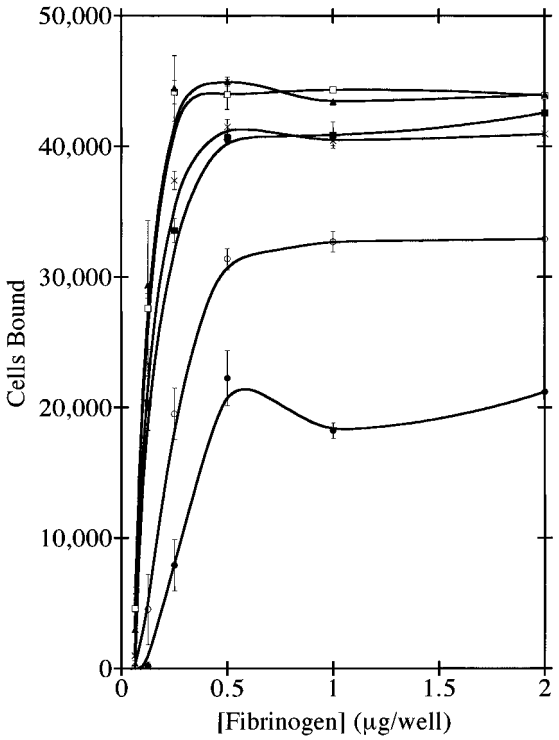

FIGURE 1: Time and dose dependency of fibroblast adhesion to fibrinogen. Fibroblasts were trypsinized and labeled for 1 h with ^{51}Cr. Labeled fibroblasts were incubated in duplicate for the indicated times in fibrinogen-coated wells: 0.5 h (●), 1 h (○), 1.5 h (×), 2 h (■), 3 h (□), and 4 h (▲). Nonadherent cells were removed and the adherent cells were counted in a γ-counter. Background adhesion to albumin-coated wells was subtracted, and the results were expressed as the average ± SD. The fibroblasts showed both time and dose dependency of adhesion to fibrinogen.

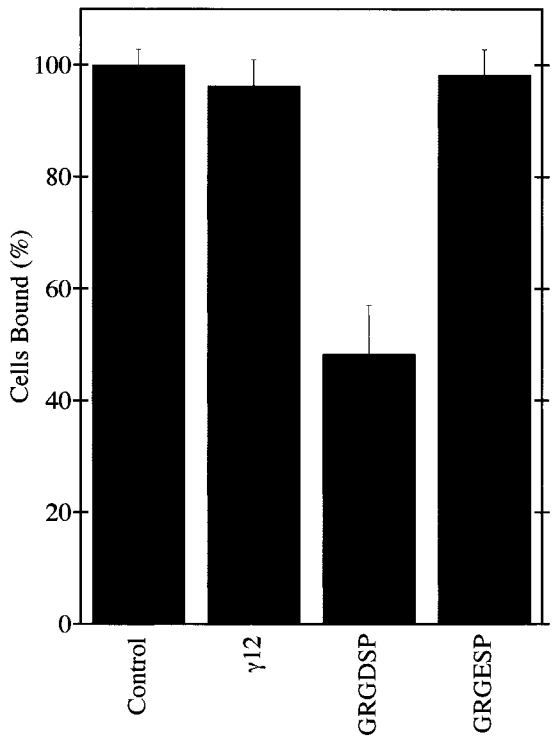

FIGURE 2: Inhibition of fibroblast adhesion by an RGD-containing peptide. Fibroblasts were trypsinized and labeled for 1 h with ^{51}Cr. Labeled fibroblasts were incubated in triplicate in the presence of 500 μM of the γ chain dodecapeptide HHLGGAKQAGDV (γ12), GRGDSP, or GRGESP for 1 h in wells coated with 1 μg/well fibrinogen. Nonadherent cells were removed, and the adherent cells were counted in a γ-counter. Background adhesion to albumin-coated wells was subtracted, and the results were expressed as the average ± SD. Only the GRGDSP peptide inhibited fibroblast adhesion to fibrinogen.

RESULTS

Characterization of Fibroblast Adhesion to Fibrinogen. The dose dependence and time course of fibroblast adhesion to fibrinogen following trypsinization were first examined. As shown in Figure 1, adhesion to fibrinogen was dose-dependent, reaching a maximum at about 1 μg of fibrinogen/well at a coating concentration of 10 μg/mL. Adhesion was also time-dependent, reaching a maximum by about 2 h. This may reflect both the time necessary for the cells to resynthesize new receptors following trypsinization as well as the time necessary for the receptors to firmly attach to fibrinogen at multiple points. As shown previously (Sporn et al., 1995), cells adhered to fibrinogen but showed no spreading during this time course (data not shown). Inhibition studies were then performed using synthetic peptide mimetics of known fibrinogen adhesion sites, particularly because previous studies had shown the involvement of RGD sites in fibroblast adhesion to fibrinogen (Tuan & Grinnell, 1989; Gailit & Clark, 1996).

Figure 2 shows that the peptide HHLGGAKQAGDV corresponding to the carboxy terminus of the γ chain, which inhibits $\alpha_{IIb}\beta_3$-mediated platelet adhesion to fibrinogen, was inactive toward fibroblast adhesion. About half of the adhesion could be inhibited using the peptide GRGDSP, whereas the related peptide GRGESP had no effect on adhesion. This partial inhibition by the GRGDSP peptide was in contrast to its effect on $\alpha_V\beta_3$-mediated endothelial cell adhesion, where >95% inhibition could be achieved (Thiagarajan et al., 1996). This raised the possibility of more than one adhesion site on fibrinogen for fibroblasts. As will be shown next, the adhesive activity consisted of at least two components, one RGD-dependent and one non-RGD-dependent.

RGD-Dependent Adhesion of Fibroblasts to Fibrinogen. In order to further investigate the role of fibrinogen's RGD sites in fibroblast adhesion, adhesion assays were performed with recombinant fibrinogens containing mutations in several possible adhesive sites. The use of highly-purified recombinant fibrinogens also minimized the potential for artifactual results from contaminating plasma fibronectin (Grinnell et al., 1980). Recombinant fibrinogens rFbgD97E and rFbgD574E were synthesized that contained RGD to RGE mutations at the α chain RGD sites from α95−97 and α572−574. In addition, rFbgγ', which has altered γ chains that lack the platelet $\alpha_{IIb}\beta_3$ binding site, was also tested for its adhesive activity. Figure 3 shows the adhesion of fibroblasts to these mutant fibrinogens. Fibroblasts showed similar adhesion to Fbg, rFbg, and rFbgγ'. Somewhat unexpectedly, neither of the RGE mutants, rFbgD97E or rFbgD574E, showed major changes in adhesive activity, even though GRGDSP peptide could partially inhibit adhesion. This is in contrast to the effect of the D574E mutation toward endothelial cell adhesion, in which adhesion to rFbgD574E is reduced by 80% (Thiagarajan et al., 1996). However, the results are similar to those obtained with keratinocytes, in which multiple sites are recognized in fibrinogen (Donaldson et al., 1994). We therefore used a double RGE mutant, rFbgD97E/D574E. Figure 4 shows that this mutant supported fibroblast adhesion, but only at higher coating concentrations than wild-type. Our interpretation of these

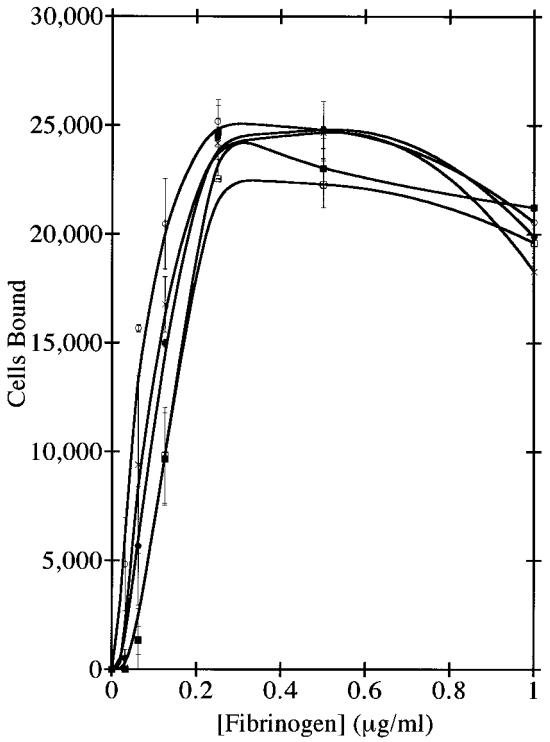

FIGURE 3: Fibroblast adhesion to recombinant fibrinogens. Fibroblasts were trypsinized and labeled for 1 h with ^{51}Cr. Labeled fibroblasts were incubated in duplicate for 1 h in fibrinogen-coated wells: Fbg (●), rFbg (○), rFbgD97E (×), rFbgD574E (□), and rFbgγ' (■). Nonadherent cells were removed and the adherent cells were counted in a γ-counter. Background adhesion to albumin-coated wells was subtracted and the results were expressed as the average ± SD. Fibroblasts adhered similarly to all these recombinant fibrinogen mutants, unlike platelets or endothelial cells.

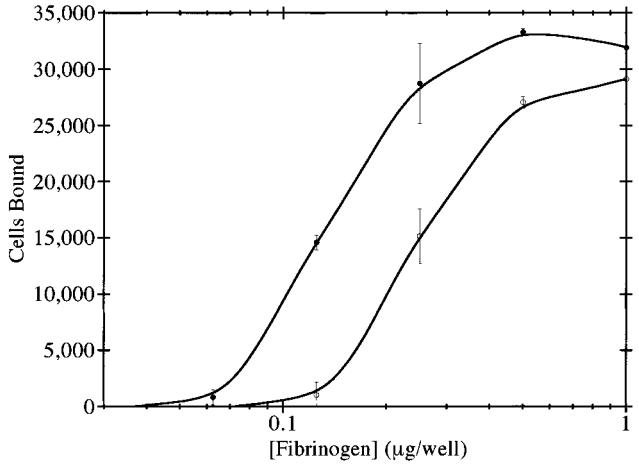

FIGURE 4: Reduced fibroblast adhesion to a double RGE fibrinogen mutant. Fibroblasts were trypsinized and labeled for 1 h with ^{51}Cr. Labeled fibroblasts were incubated in duplicate for 1 h in fibrinogen-coated wells: rFbg (●), rFbgD97E/D574E (○). Nonadherent cells were removed, and the adherent cells were counted in a γ-counter. Background adhesion to albumin-coated wells was subtracted, and the results were expressed as the average ± SD. The double RGE mutant mediated fibroblast adhesion, but required higher coating concentrations than wild-type recombinant fibrinogen.

results is that the RGD sites in fibrinogen can be recognized by fibroblasts, but at least one other adhesive site can also mediate adhesion.

The involvement of α_V-containing integrins as the source of the RGD-dependent adhesion was investigated. Previous studies had shown that α_V integrins play a significant role in fibroblast adhesion to fibrinogen (Gailit & Clark, 1996).

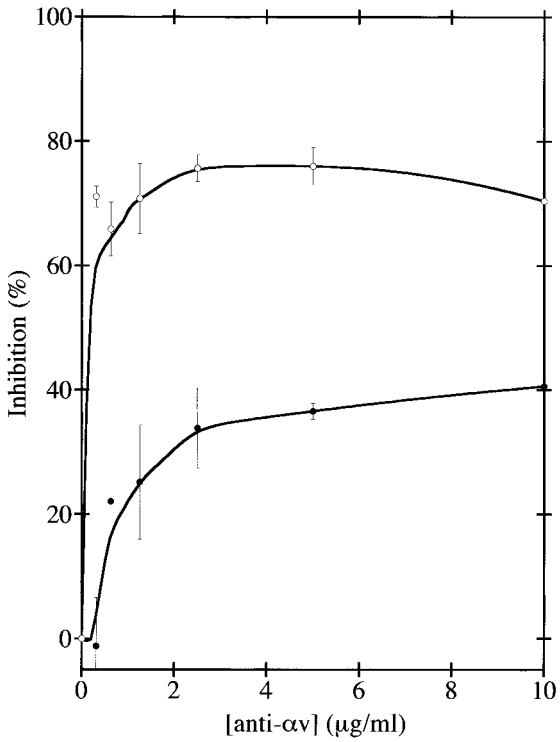

FIGURE 5: Inhibition of fibroblast and HMEC-1 adhesion by anti-α_V. Fibroblasts (●) and HMEC-1 cells (○) were trypsinized and labeled for 1 h with ^{51}Cr. Labeled cells were incubated in duplicate in the presence of the indicated amounts of a monoclonal antibody against integrin α_V for 1 h in wells coated with 1 μg/well fibrinogen. Nonadherent cells were removed, and the adherent cells were counted in a γ-counter. Background adhesion to albumin-coated wells was subtracted, and the results were expressed as the average ±SD. The antibody inhibited most of the HMEC-1 adhesion, but less than half of the fibroblast adhesion.

Consistent with the RGD inhibition data, about half of the fibroblast adhesion to fibrinogen could be inhibited by the α_V monoclonal antibody (Figure 5). In contrast, the α_V monoclonal antibody almost completely inhibited fibrinogen adhesion of a control microvascular endothelial cell line, HMEC-1 (Ades et al., 1992), similar to RGD peptide inhibition (Figure 6). Together, these data demonstrate the contribution of α_V integrins in fibroblast adhesion to fibrinogen.

RGD-Independent Adhesion of Fibroblasts to Fibrinogen. The source of the RGD-independent adhesion was further investigated. The peptide GPRP, which mimics the neo-epitopes that are exposed by thrombin cleavage of fibrinopeptides A and B, had little effect on fibroblast adhesion, suggesting that the fibroblasts were not adhering through fibrin-specific epitopes (Figure 6). It should be emphasized that this result does not imply that fibroblasts cannot adhere to the fibrin-specific epitopes, only that they are not exposed under these assay conditions. Monoclonal antibodies that block adhesion to either the α_M or β_2 subunits of $\alpha_M\beta_2$ had no effect on fibroblast adhesion to fibrinogen (data not shown), consistent with the lymphocyte-restricted expression of the $\alpha_M\beta_2$ integrin.

The involvement of ICAM-1 in the RGD-independent adhesion was investigated. The γ3 peptide NNQKIVNLKEKVAQLEA, which mimics the adhesive site for ICAM-1 on the γ chain, inhibited about half of the fibroblast adhesion to fibrinogen (Figure 6). In contrast, the γ3 peptide had little effect on HMEC-1 adhesion to fibrinogen. This again is

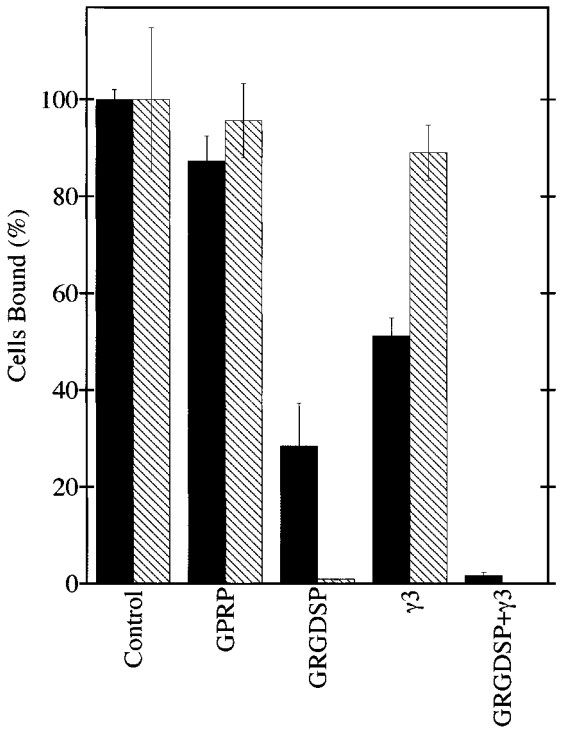

FIGURE 6: Inhibition of fibroblast and HMEC-1 adhesion by RGD and γ3 peptides. Fibroblasts (■) and HMEC-1 cells (□) were trypsinized and labeled for 1 h with ^{51}Cr. Labeled cells were incubated in triplicate in the presence of 500 μM of GPRP, GRGDSP, or the γ3 peptide NNQKIVNLKEKVAQLEA for 1 h in wells coated with 1 μg/well fibrinogen. Nonadherent cells were removed, and the adherent cells were counted in a γ-counter. Background adhesion to albumin-coated wells was subtracted, and the results were expressed as the average ± SD. The inhibition of fibroblast adhesion by the combined GRGDSP and γ3 peptides was essentially complete.

consistent with the finding that the majority of endothelial cell adhesion to fibrinogen is mediated by the RGD-dependent integrin $\alpha_V\beta_3$. Furthermore, the combination of this peptide with the GRGDSP peptide virtually abolished fibroblast adhesion to fibrinogen, suggesting that both α_V and ICAM-1 may contribute to fibroblast adhesion to fibrinogen.

DISCUSSION

Early studies of rodent fibroblastic cell line adhesion to fibrinogen led to the conclusion that normal wound healing could not occur in the absence of fibronectin because fibroblasts could not adhere to the fibrin clot (Grinnell et al., 1980). The present studies and those of others (Brown et al., 1993; Sporn et al., 1995; Gailit & Clark, 1996) using normal human dermal fibroblasts demonstrate that these fibroblasts do indeed adhere to fibrin(ogen). The results from the present experiments show that fibroblasts can recognize multiple epitopes in fibrinogen. This bears some similarity to the results obtained with keratinocytes (Donaldson et al., 1994) in which several epitopes in fibrinogen could be used for chemotaxis.

The RGD-dependent adhesion of fibroblasts to fibrinogen appears to be mediated primarily by α_V integrins. This is not unexpected, since this integrin subunit is one of only two integrin α subunits that has been shown to bind fibrinogen in non-lymphoid cells (Gailit & Clark, 1996), the other being α_{IIb} on platelets (Phillips & Agin, 1977). The RGD-independent adhesive component appears to be mediated by ICAM-1. The complete and additive inhibition of fibroblast adhesion by the GRGDSP and γ3 peptides suggests the involvement of both α_V integrins and ICAM-1.

Several investigations have shown that conversion of fibrinogen to fibrin by thrombin exposes new cellular binding sites (Chalupowicz et al., 1995; Sporn et al., 1995). In particular, the exposure of the β15−42 sequence leads to interactions with an endothelial cell surface receptor (Erban & Wagner, 1992) and exposes a new heparin binding site (Odrljin et al., 1996). There is also substantial evidence that cross-linking of fibrin by factor XIII plays a major role in fibroblast adhesion and migration in fibrin clots (Beck et al., 1961; Grinnell et al., 1980; Brown et al., 1993). Fibrinogen can also bind to cellular fibronectin on fibroblasts (Colvin et al., 1979). Fibrinogen binding to several different fibroblastic cell types correlates with fibronectin binding. In addition, proteolytically-digested fibrinogen fraction I-9, that lacks the fibronectin binding site at the carboxy terminus of the α chain, binds much less to fibroblasts than intact fibrinogen. Unfortunately, the region of the α chain lacking in I-9 fibrinogen also contains the RGD binding epitope for α_V integrins, so that either defective fibronectin binding, α_V binding, or both could account for the results. However, antibodies to fibronectin were unable to inhibit fibrinogen adhesion to fibroblasts (Colvin et al., 1979), demonstrating that fibrinogen can also bind to fibroblasts through sites that do not involve fibronectin.

The multiplicity of potential fibroblast adhesion sites on fibrin(ogen) has made the determination of the role(s) of such adhesion sites problematic. It may be that each of these sites plays a different role in fibroblast adhesion, either temporally or functionally. For example, the expression of RGD-independent adhesion following trypsinization that was observed in the present study was delayed compared to RGD-dependent adhesion (data not shown), suggestive of different temporal roles. Furthermore, different α_V integrins are known to participate in different intracellular signaling events (Friedlander et al., 1995), indicating different functional roles. It is likely that, during wound healing, each of these adhesive sites plays a particular role at a particular time. The recombinant fibrinogens described in this study may prove useful in dissecting the contribution of each of the different adhesion sites to wound healing.

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