Suppression of p53 Activity and p21\textsuperscript{WAF1/CIP1} Expression by Vascular Cell Integrin \(\alpha v\beta 3\) during Angiogenesis

Staffan Strömland, Jürgen C. Becker, Mayra Yebra, Peter C. Brooks, and David A. Cheresh

Departments of Immunology and Vascular Biology, IMM24, The Scripps Research Institute, La Jolla, California 92037

Abstract

Induction of p53 activity in cells undergoing DNA synthesis represents a molecular conflict that can lead to apoptosis. During angiogenesis, proliferative endothelial cells become apoptotic in response to antagonists of integrin \(\alpha v\beta 3\) and this leads to the regression of angiogenic blood vessels, thereby blocking the growth of various human tumors. Evidence is presented that administration of \(\alpha v\beta 3\) antagonists during angiogenesis in vivo selectively caused activation of endothelial cell p53 and increased expression of the p53-inducible cell cycle inhibitor p21\textsuperscript{WAF1/CIP1}. In vitro studies revealed that the ligation state of human endothelial cell \(\alpha v\beta 3\) directly influenced p53 activity and the bax cell death pathway. Specifically, agonists of endothelial cell \(\alpha v\beta 3\), but not other integrins, suppressed p53 activity, blocked p21\textsuperscript{WAF1/CIP1} expression, and increased the bcl-2/bax ratio, thereby promoting cell survival. Thus, ligation of vascular cell integrin \(\alpha v\beta 3\) promotes a critical and specific adhesion-dependent cell survival signal during angiogenesis leading to inhibition of p53 activity, decreased expression of p21\textsuperscript{WAF1/CIP1}, and suppression of the bax cell death pathway. (J. Clin. Invest. 1996, 98:426–433). Key words: endothelial cells \cdot gene expression \cdot apoptosis \cdot cell cycle \cdot bcl-2

Introduction

Angiogenesis is a critical process during development, wound healing, and various diseases including cancer, adult blindness, and inflammatory disorders (1). We recently showed that integrin \(\alpha v\beta 3\) becomes expressed on angiogenic vascular cells where it plays a critical role in angiogenesis induced by basic Fibroblast growth Factor (bFGF), TNF-\(\alpha\), or fragments of human tumors in the chick embryo (2, 3). In fact, antagonists of \(\alpha v\beta 3\) administered intravenously to chick embryos caused regression of angiogenic blood vessels on the chorioallantoic membrane (CAM) due to unscheduled apoptosis (2). Importantly, this resulted in the regression of human tumors on the chick CAM or in human skin transplants on SCID mice (2, 4).

Cell survival and proliferation depend on appropriate signals mediated by growth factors and/or adhesion proteins within the extracellular matrix (5, 6). However, incompatible signaling events can result in apoptosis (7–12). We hypothesized that during angiogenesis, integrin \(\alpha v\beta 3\) promotes an adhesion-dependent survival signal necessary for normal cell cycle progression, since antagonists of \(\alpha v\beta 3\) administered in vivo preferentially caused apoptosis of proliferating vascular cells (2). Therefore, we examined whether the observed apoptosis might be associated with the induction of conflicting signals during endothelial cell cycle progression. Recent reports have demonstrated that cells expressing active p53 while undergoing DNA synthesis in vitro become apoptotic, presumably due to conflicting signals (7–11). Induction of apoptosis by p53 has been associated with its ability to promote or repress transcription of genes that either influence cell cycle progression or directly regulate cell survival and apoptosis (13–16). Therefore, experiments were designed to evaluate whether ligation of endothelial cell \(\alpha v\beta 3\) influenced expression and/or activity of p53 and of putative mediators of p53-induced apoptosis during angiogenesis.

Methods

Cell lines and tissue culture. Human umbilical vein endothelial cells (HUVECs) from pooled donors (Clonetics Corp., San Diego, CA) were grown in M199 containing 20% FBS, 100 \(\mu\)g/ml gentamicin, 4 mM l-glutamine, 0.9 mg/ml heparin and 30 \(\mu\)g/ml endothelial cell growth supplement (Upstate Biotech. Inc., Lake Placid, NY).

Cell adhesion to antiintegrin antibodies as agonists of integrin function. For evaluation of effects by individual integrins, plastic dishes were first coated with 25 \(\mu\)g/ml goat anti–mouse polyclonal antibodies (Jackson ImmunoResearch Labs., Inc., West Grove, PA) at 37°C for 2 h, followed by blocking with 1% heat-denatured BSA at 37°C for 1 h. Then, mouse monoclonal antibodies (10 \(\mu\)g/ml) LM 609 (anti–\(\alpha v\beta 3\)), P1F6 (anti–\(\alpha v\beta 5\)), or P4C10 (anti–\(\beta 1\)) were allowed to bind the immobilized anti–mouse antibody for 14 h at 4°C. Subconfluent (∼50%) HUVEC cells were harvested with EDTA, washed, and resuspended in 20 mM Hepes, 140 mM NaCl, 5 mM KCl, 1 mM CaCl\(_2\), 2 mM MgCl\(_2\), 5 mM glucose, and 5 mM sodium pyruvate, pH 7.4 (adhesion buffer). Cells were then allowed to attach to antibody-coated dishes for 4 h at 37°C. To maintain cells in suspension, some dishes were coated only with 1% heat-denatured BSA.

Chick CAM angiogenesis assay. 10-d-old chick embryos, purchased from McIntyre Poultry (Lakeside, CA), were incubated at 37°C with 60% humidity. Filter discs (Whatman Inc., Clifton, NJ), 1 \(\times\) 1 cm, were saturated with 1 \(\mu\)g/ml bFGF (Genzyme Corp., Cambridge, MA) and placed on top of an avascular zone of the CAM as previously described (3). After 20–24 h, at the peak of \(\alpha v\beta 3\) expression (S. Strömland, and D.A. Cheresh, unpublished results), embryos were injected intravenously with 100 \(\mu\)l PBS containing antagonists to \(\alpha v\beta 3\), 300 \(\mu\)g mAb LM 609 or 100 \(\mu\)g peptide 66203 (cyclo-RGDKV). Control embryos received 300 \(\mu\)g mAb CSAT (anti–\(\beta 1\)) or 100 \(\mu\)g peptide 69601 (cyclo-RADKV). 48 h later, CAM tissues were injected intravenously with 100 \(\mu\)l of either cyclo-RGDKV or cyclo-RADKV. The histological changes were analyzed.

Received for publication 15 February 1996 and accepted in revised form 23 April 1996.

1. Abbreviations used in this paper: bFGF, basic fibroblast growth factor; CAM, chorioallantoic membrane; EMSA, electrophoretic mobility shift assay; HUVEC, human umbilical vein endothelial cells; TNA, total nucleic acids.

J. Clin. Invest. © The American Society for Clinical Investigation, Inc. 0021-9738/96/07/0426/08 $2.00
Volume 98, Number 2, July 1996, 426–433

426  S. Strömland, J.C. Becker, M. Yebra, P.C. Brooks, and D.A. Cheresh
Isolation of CAM cells and sorting of endothelial cells. After induction of angiogenesis, CAMs were resected and washed three times in sterile PBS, finely minced, and resuspended in 0.25% bacterial collagenase (Worthington Biochemical Corp., Freehold, NJ). After incubation for 120 min at 37°C with occasional vortexing, cells were washed three times in PBS with 1% BSA, and then incubated with a 1:100 dilution of antiFactor VIII rabbit polyclonal antibodies 016P (BioGenex Labs., San Ramon, CA) at room temperature for 60 min. After three washes in PBS/1% BSA, cells were incubated with FITC-conjugated goat anti-rabbit antibodies at 1:50 dilution (Biosource International, Camarillo, CA) for 45 min at 25°C. Cells were then washed three times (PBS/1% BSA) and positive staining cells were separated from negative by flow cytometry using a FACStar® cell sorter (Becton Dickinson & Co., Mountain View, CA) with Consort 30 software (Becton Dickinson & Co.). Cells incubated with the secondary antibody were used to set negative markers and the settings typically yielded 15–20% positive cells.

Preparation of nucleic acids and mRNA analyses. Total nucleic acids (TNA) were isolated by the method of Durum and Palmeter (17) using 3–5 pooled CAMs per preparation. The TNA concentrations were measured by spectrophotometry at 260 nm. Chicken bcl-2 mRNA and β-actin mRNA was quantified by solution hybridization where 50,000 cpm of a 35S-CTP-labeled (Amersham Corp., Arlington Heights, IL) probe was hybridized to 1–2 μg (β-actin) or 10–20 μg (bcl-2) of the TNA samples as previously described (18) with reagents from Promega Biotech, Inc. (Madison, WI). The bcl-2 clone was a 532-bp Aval-BamHI (from 71 to 603) cDNA fragment of the chicken bcl-2 (19), subcloned into a pGEM™ 4Z riboprobe vector (Promega Biotech. Inc.). β-Actin mRNA was quantified using a 2.2-kb cDNA coding for chicken β-actin (20), subceloned into pGEM™ 3. The hybridization signals were compared with that of a standard curve produced using synthetic mRNA strands complimentary to the probes made from the same cDNAs. Three TNA preparations were analyzed for each group and the values were determined using four incubations for each sample at two different concentrations, to ensure that the analyses were within a linear range.

Western blot analyses. Cells or tissues were lysed in a modified RIPA buffer (150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA, 1% Triton X-100, 1% deoxycholic acid, 1 mM PMSF, with addition of Complete™ protease inhibitor cocktail [Boeringer Mannheim, Mannheim, Germany]). Protein concentrations were determined by a BCA assay (Pierce Chemical Co., Rockford, IL) using BSA as a standard. Cell lysates from three to four pooled CAMs or from HUVEC cells (30 μg protein) were run in an 8% (p53) or 12% (all others) SDS-molecular weight size markers (Amersham Corp.) and allowed to swell on ice for 15 min; after which, 25 μl 10% Nonidet P-40 was added and the tubes extensively vortexed. After centrifugation, the nuclear pellet was resuspended in 80 μl ice cold buffer B (20 mM Hepes, pH 7.9, 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF) and vigorously rocked at 4°C for 15 min. Nuclear extracts were cleared by centrifugation. Protein content was assayed with Bradford reagent (Bio-Rad Laboratories).

3P-end-labeled double-stranded oligonucleotides containing the following human recognition sequences were used: p53 (5′ CAG TGT CTA CAG GCA AAG GCA TGT CTG 3′) and SP1 (5′ ATT CGA TCG GGG GGC GAC GAC 3′). An oligonucleotide containing a mutated p53 recognition sequence (5′ CAT CAA TGT CTA CAG GCA AAG GCA TAC GTG 3′) served as a control to measure nonspecific binding of proteins present in the nuclear extracts. Each reaction mixture contained 10 fmol probe and 5 μg protein from nuclear extracts. Binding reactions were conducted in 50 mM Hepes, 30% glycerol, 50 mM KCl, 10 mM MgCl2, 1 mM EDTA, 1 mM DTT, and 1 mg poly(dI-dC) in a final volume of 15 μl. These reactions were allowed to occur at 4°C for 20 min. For supershift experiments, nuclear extracts were preincubated with 1 μg of anti-p53 monoclonal antibody 421 (Oncogene Science Inc.) for 20 min at 4°C. Complexes were resolved on 6% nondenaturing polyacrylamide gels in 50 mM Tris, 45 mM borate, and 0.5 mM EDTA, pH 7.4. The samples were then subjected to electrophoresis for 2 h at 10V/cm. Gels were dried and subsequently visualized with a PhosphorImager (Molecular Dynamics, Inc.).

Results

Experiments were designed to investigate the role of integrin αvβ3 in the regulation of vascular cell survival during angiogenesis. Angiogenesis was induced with bFGF on the chick CAM of 10-d-old chick embryos. 1 d later embryos were injected intravenously with anti-αvβ3 monoclonal antibody (mAb LM609) or a control antibody directed to chick β1 integrins (mAb CSAT). Administration of anti-αvβ3 specifically leads to extensive apoptosis among proliferating endothelial cells on these CAMs within 48 h (6). To evaluate whether the apoptosis observed was associated with induction of p53 activity, nuclear extracts prepared from these CAMs were examined for p53 binding activity in a gel mobility shift assay. Administration of a single intravenous dose of anti-αvβ3, sufficient to induce apoptosis after bFGF stimulation (2), caused a marked increase in p53 DNA binding activity as measured by the mobility shift (Fig. 1 A). However, CAMs examined from embryos injected with anti-β1 showed negligible apopto-osis (2) and p53 activity (Fig. 1 A). The induction of p53 activity with anti-αvβ3 was specific since excess unlabeled oligonucleotide effectively competed for this binding activity (Fig. 1 A) and a mutant oligonucleotide failed to cause the gel shift (data not shown). Also, injection of anti-αvβ3 produced no change in SP1 binding activity (Fig. 1 A).

To determine whether this increased p53 activity was associated with endothelial cells within these tissues, single cell suspensions isolated from CAMs by collagenase treatment were stained for the endothelial cell marker, Factor VIII, and were sorted by flow cytometry. Analysis of nuclear extracts from these CAM-sorted endothelial cells revealed increased p53 binding activity after exposure to both bFGF and anti-αvβ3 while SP1 binding activity remained unchanged (Fig. 1 B). In contrast, injection of mAb CSAT had no effect on p53 or SP1
S. Strömblad, J.C. Becker, M. Yebra, P.C. Brooks, and D.A. Cheresh

activity, consistent with its inability to affect angiogenesis or apoptosis (2). The observed gel shifts for p53 and SP1 were abolished with excess unlabeled oligonucleotide (data not shown). These findings demonstrate that administration of antagonists in vivo promote apoptosis and p53 activity among angiogenic endothelial cells.

Figure 1. Regulation of p53 activity during bFGF-induced angiogenesis on the chick CAM. (A) Angiogenesis was stimulated with bFGF on the chick CAM in the presence of anti-αvβ3 or anti-β1. Nuclear extracts from three pooled CAMs were analyzed by an EMSA, examining p53 or SP1 DNA binding activity as described in Methods (left). In parallel, the nuclear extract from the anti-αvβ3 treated CAM was incubated with a 5- or 50-fold excess of unlabeled oligonucleotide (right). (B) Angiogenesis was stimulated on the CAM with bFGF for 3 d in the presence of anti-αvβ3 or anti-β1. Factor VIII-positive cells (endothelial cells) were then sorted out from a single cell suspension from six to ten pooled CAMs using a FACS® sorter as described in Methods. Nuclear extracts from the sorted endothelial cells were subsequently analyzed by p53 or SP1 EMSAs.

Figure 2. Regulation of p21WAF1/CIP1 during bFGF-induced angiogenesis. Angiogenesis was stimulated on the CAM with bFGF for 3 d in the presence of anti-αvβ3 antibodies, αvβ3 antagonist cyclic peptide RGDfV, anti-β1 antibodies or control cyclic peptide RADfV. Lysates from six to ten pooled CAMs (15 μg protein) (top) or endothelial cells derived from these CAMs by FACS sorting (8 μg protein) (bottom) were analyzed for p21WAF1/CIP1 protein levels by Western blotting as described in Methods.
endothelial cells were plated on the nonadhesive substrate BSA under serum-containing growth medium (data not shown). However, when activity among adherent, proliferative cells maintained in serum activity. As expected, there was little if any detectable p53 activity, suggesting this particular adhesion event suppressed p53 activity. These results support the in vivo findings that integrin αvβ3 ligation specifically influences the functional activity of p53. Importantly, regulation of p53 was independent of cell shape and actin assembly since immobilized anti-β1 caused cell spreading yet failed to influence p53 activity. Once again, the specificity of the p53 gel shift was shown by the failure of a mutant oligonucleotide to induce a shift (data not shown), inhibition with excess unlabeled oligonucleotide, and the fact that SP1-binding activity was identical on all substrates (Fig. 4 A). In addition, the p53 activity was identified in an antibody-mediated supershift assay (Fig. 4 B). Surprisingly, the observed change in p53 DNA binding activity could not be explained by an altered level of p53 protein in these cells since p53 protein levels remained constant under all adhesion conditions (Fig. 4 B). These findings demonstrate that ligation of endothelial cell αvβ3 is sufficient to inhibit p53 activity without influencing its expression. Furthermore, in support of the in vivo findings, αvβ3 ligation promotes decreased p21WAF1/CIP1 levels in these cells, revealing the association between this cell cycle suppressor and the activity of p53 during αvβ3-mediated adhesion of endothelial cells (Fig. 5). Thus, the ligation state of endothelial cell αvβ3 regulates p53 activity and the expression of p21WAF1/CIP1 both in vitro and in vivo, which may account for the role of αvβ3 in vascular cell survival (2).

The role of p53 in promoting apoptosis has also been linked to its ability to repress transcription of bcl-2 while activating that of bax (14–16). Bcl-2 is known to potentiate cell survival based on its ability to dimerize with the death-promoting molecule bax (31–32). Therefore, cell survival is favored by a high bcl-2/bax ratio. To examine the role of endothelial cell αvβ3 ligation on the bcl-2/bax ratio, cells were allowed to attach to immobilized antiintegrin antibodies as described above. While αvβ3 and β1 integrin ligation caused increased bcl-2 expression relative to nonadherent cells, only αvβ3 produced a concomitant decrease in bax expression, resulting in a sharp increase in the bcl-2/bax ratio (Fig. 6). These findings provide further support that ligation of endothelial cell αvβ3 provides a distinct adhesion-dependent survival signal and supports our in vivo observations that antagonists of this integrin selectively promote endothelial cell apoptosis during angiogenesis. Consistent with this hypothesis is the fact that administration of antibody or peptide antagonists of αvβ3 during chick CAM angiogenesis blocks bFGF-induced bcl-2 mRNA and protein expression on the CAM as measured by solution hybridization and Western blot analyses (Fig. 7).

**Discussion**

Integrin-mediated cell attachment regulates cell survival and proliferation in several cell types in vitro (2, 5, 6, 12, 26–30). Also, cell attachment to extracellular matrix proteins in vitro is capable of regulating the apoptosis-related genes interleukin-1-β-converting enzyme and bcl-2 (29, 30), as well as the cell cycle inhibitors p21WAF1/CIP1 and p27KIP1 (33). However, in vivo cells typically use multiple integrins for attachment to a wide variety of adhesive proteins. Thus, it remains unclear as to how individual matrix proteins or integrin receptors impact the cell survival within the context of a physiologically relevant extracellular matrix. To this end, we observed that integrin αvβ3, whose expression is enhanced on angiogenic vascular cells, promotes a survival signal since antagonists of this integrin...
cause unscheduled apoptosis of newly forming blood vessels (2). This results in the disruption of ongoing angiogenesis or neovascularization in the quail embryo, chick CAM, rabbit eye, or human skin transplants on the SCID mouse (2–4, 34, 35). In fact, this leads to either prevention of human tumor growth or regression of preexisting tumors in the chick CAM or in human skin transplanted on SCID-mice (2, 4).

Integrin αvβ3 can recognize several extracellular matrix proteins, including vitronectin, fibronectin, osteopontin, von Willebrand factor, fibrinogen, and proteolyzed collagen (27, 36, 37). At present, it remains unclear as to which of the αvβ3-directed ligand(s) promotes vascular cell survival during angiogenesis. However, recent work in our laboratory demonstrates that the matrix metalloproteinase-2 directly binds to αvβ3 and thereby associates with the surface of angiogenic vascular cells (38). Thus, matrix metalloproteinase-2 once bound to αvβ3, may serve to promote endothelial cell invasion while providing a survival factor, i.e., proteolyzed collagen which binds to αvβ3, thereby facilitating angiogenesis. In support of this contention, αvβ3 recognition of proteolyzed collagen has been shown to promote survival of melanoma cells in vitro (27).

Based on the fact that apoptosis was induced selectively in proliferating vascular cells upon treatment with antagonists to αvβ3 (2), we hypothesized that ligation of αvβ3 during angiogenesis was required for normal cell cycle progression. We found that ligation of αvβ3 during angiogenesis suppresses the expression and/or activity of p53 and the p53-inducible cell cycle inhibitor p21WAF1/CIP1. Presence of either p53 or p21WAF1/CIP1 in proliferating cells has been found to induce apoptosis (7–11, 12). Thus, it appears that molecules that can promote cell cycle arrest cause apoptosis under other circumstances. The induction of apoptosis during proliferation is presumably caused by

Figure 4. Integrin regulation of endothelial cell p53 activity. (A) Nuclear extracts were prepared from HUVECs attached to immobilized mAbs LM 609 (anti–αvβ3), P4C10 (anti–β1), or P1F6 (anti–αvβ3) or maintained in suspension by BSA blocking for 4 h. Nuclear extracts were then used in p53 or SP1 EMSAs as described in Methods (left). HUVECs grown in serum-containing media were used as control. Extracts from HUVECs denied attachment were incubated with the 32P-labeled probe in the presence of 5- or 50-fold excess of unlabeled oligonucleotide (right). (B) (Top) Nuclear extract prepared from HUVECs that were prevented from adhering with heat-denatured BSA was analyzed in a super shift gel assay after preincubation in the presence or absence of anti–p53 mAb 421. (Bottom) Lysates (15 μg) from HUVECs attached to various immobilized antiintegrin antibodies were analyzed for p53 protein levels by Western blot analysis as described in Methods.
incompatible molecular signals when stimulation of DNA synthesis occurs concurrent with growth arrest signals.

Furthermore, antagonists of αvβ3 administered during angiogenesis specifically prevented an increase in the bcl-2 levels, consistent with the role of p53 as a transcriptional repressor of bcl-2 (14, 15) and with the induction of vascular cell apoptosis. This is consistent with previous studies showing that lack of attachment to the extracellular matrix results in apoptosis of various cell types (2, 12, 26–30, 39, 40). It has been suggested that this phenomenon is caused by the lack of attachment per se (28), but other studies, including data presented here, clearly point out that cell survival is mediated through distinct signals from specific integrins and in some cases also requires a three-dimensional matrix (2, 12, 27, 29, 30). However, various integrins can serve to promote cell survival. For example, distinct β1-integrins can mediate cell survival in mammary cells and Chinese hamster ovary cells in vitro, whereas αvβ3 can mediate melanoma cell survival in three-dimensional collagen and survival of proliferating vascular cells in vivo (2, 12, 27, 29, 30). The integrin required for cell survival and the integrin-dependent survival signals might thus be cell type and condition specific.

While the use of receptor antagonists in vivo can suggest receptor function, we performed experiments to directly examine the effect of αvβ3 ligation on cultured human endothelial cells. In this case, antiintegrin antibodies were immobilized on a substrate facilitating their use as agonists of integrin function, thereby promoting adhesion of cells via clustering of a given integrin. This approach has been successfully used to examine integrin-specific signaling events in the absence of growth factors and other adhesion events, also demonstrating that distinct integrins mediate different signals (25, 41, 42).

Inhibition of intercellular contact in a colon carcinoma cell line in vitro, mediated by an undefined αv integrin, induces relocalization of p53 to the nucleus (39). However, it is unclear if this translocation is associated with any change in p53 DNA binding activity. We here show that αvβ3-mediated endothelial cell adhesion selectively reduces p53 activity and p21WAF1/CIP1 levels, since attachment via anti–β1 integrins or αvβ5 does not. Furthermore, the degree of cell spreading has been suggested as an important factor for endothelial cell survival (40). However, we demonstrate that attachment to either anti–αvβ3 or anti–β1 resulted in equivalent cell spreading, yet these substrates differed in their ability to regulate p53 activity and p21WAF1/CIP1 expression. Ligation of αvβ3 also resulted in a sharp increase in the bcl-2/bax ratio, consistent with the role of p53 as a transcriptional repressor of bcl-2 and an activator of bax (14–16). Based on the fact that bcl-2 potentiates cell survival by forming heterodimers with the death promoting molecule bax (31, 32), the increased bcl-2/bax ratio is expected to directly promote endothelial cell survival.

These studies provide a mechanism explaining how antagonists of αvβ3 can block angiogenesis and thereby cause regression of human tumor growth (2, 4). Experiments reported here demonstrate that αvβ3 ligation induces a distinct endothelial
signals, thereby facilitating the proliferation and maturation of the suppression of apoptosis and of conflicting growth arrest.

References


Acknowledgments

We thank Dr. Alfred Jonczyk (Merck, KGaA, Darmstadt, Germany) for providing cyclic peptide antagonists of αvβ3. We also thank Drs. John C. Reed, Nancy Boudreau, and Steven L. Reed for helpful discussions. This is manuscript number 9821-IMM from The Scripps Research Institute.

This study was supported by grants HI-54444, CA50286, and CA45726 from the National Institutes of Health (NIH) and a faculty research award from the American Cancer Society (D.A. Cheresh). S. Stromblad was supported by postdoctoral fellowships from the Swedish Cancer Society and the Wenner-Gren Foundation. M. Yebra and P. Brooks were supported by NIH training grants T32 AI 07244.

References


Figure 7. Regulation of bcl-2 during chick CAM angiogenesis. (A) Angiogenesis was stimulated by bFGF in the chick CAM for 3 d in the presence or absence of mAb LM609 (anti-αvβ3) and bcl-2 mRNA and β-actin mRNA levels were measured by solution hybridization as described in Methods. Each bar represents the mean of three TNA preparations±SD. Each preparation was from four to five pooled CAM tissues. (B) Angiogenesis was stimulated as above in the presence of mAb LM 609 (anti-αvβ3) or mAb CSAT (anti-β1) (left) or in the presence of cyclic RGD (anti-αvβ3) or RAD (control) peptides (right). CAM tissues were collected and cell lysates of four to five pooled CAMs were analyzed for bcl-2 protein levels by Western blotting under nonreducing conditions as described in Methods. The intensity of the band representing bcl-2 (indicated) was then quantified by densitometry (bars). Bars represent the mean values of three separate experiments±standard error.


