Leukocyte adhesion during hypoxia is mediated by HIF-1-dependent induction of β_2 integrin gene expression

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Inflammatory responses are associated with significant changes in tissue metabolism. In particular, metabolic shifts during inflammation can result in significant tissue hypoxia, with resultant induction of hypoxia-responsive genes. Given this association, we hypothesized that leukocyte functional responses are influenced by hypoxia. Initial experiments revealed that exposure of the promonocytic cell line U937 to hypoxia resulted in increased adhesion to activated endothelia. Such increases were transcription-dependent and were blocked by antibodies directed against β_2, but not β_1, integrins. Analysis of β_2 integrin mRNA and protein in U937 cells revealed a 5- to 6-fold increase with hypoxia. Extension of this analysis to hypoxic human whole blood revealed prominent induction of β_2 integrin mRNA and protein *ex vivo*. Furthermore, murine β_2 integrin mRNA was found to be significantly induced during hypoxia *in vivo*. Subsequent studies identified a binding site for hypoxia-inducible factor 1 (HIF-1) in the CD18 gene. This gene encodes the subunit common to all four known types of β_2 integrin heterodimer. HIF-1 binding was demonstrated *in vivo*, and mutational analysis of the HIF-1 site within the CD18 promoter resulted in a loss of hypoxia inducibility. Taken together, these results demonstrate that hypoxia induces leukocyte β_2 integrin expression and function by transcriptional mechanisms dependent upon HIF-1.

Inflammatory lesions are characterized by hypoxia and the dramatic recruitment of myeloid cells (1–4). However, little is known about how hypoxia directly influences myeloid function at the molecular level. The recruitment of myeloid cells to sites of inflammation is coordinated by the β_2 integrin family of adhesion receptors (5, 6). The β_2 integrins are heterodimeric glycoproteins that exist in four forms. Each form is composed of a unique α-subunit, encoded by the CD11a, CD11b, CD11c, or CD11d gene, noncovalently associated with a common β-subunit encoded by the CD18 gene (7, 8). The importance of these receptors is best demonstrated in the genetic disorder leukocyte adhesion deficiency. There, patients lack normal expression of the CD18 β-subunit and, as a result, show increased susceptibility to infection due to abnormal leukocyte function (9, 10). Here, we report that hypoxia directly influences myeloid function by inducing β_2 integrin expression. Furthermore, we demonstrate that this induction is mediated by the heterodimeric transcription factor hypoxia-inducible factor-1 (HIF-1) (11–15).

Methods

Cell Culture. The human myelocytic cell line U937 was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured as described (16). U937 cells were exposed to hypoxia by replacing the growth medium with fresh media equilibrated with a hypoxic gas mixture and incubation in a hypoxic chamber (Coy Laboratory Products, Ann Arbor, MI). Measured partial pressure of O_2 (pO_2) was 20 mmHg, with the balance made up of nitrogen, carbon dioxide (ambient 5% CO_2), and water vapor. Human microvascular endothelial cells (HMEC-1) were a kind gift of Francisco Candal (Centers for Disease Control, Atlanta, GA) (17–19). Confluent monolayers of HMEC-1 were activated by 100 ng/ml lipopolysaccharide (List Biological Laboratories, Campbell, CA).

Immunoprecipitation. U937 cells were exposed to experimental conditions and washed with Hanks' balanced salt solution (HBSS); then, their extracellular cell surface proteins were labeled with biotin (20). Unbound biotin was quenched with 50 mM NH_4Cl, and cells were lysed in a buffer containing 150 mM NaCl, 25 mM Tris, 1 mM $MgCl_2$, 1% Triton X-100, 1% Nonidet P-40, 5 mM EDTA, 5 μg/ml chymostatin, 2 μg/ml aprotinin, and 1.25 mM PMSF (Sigma–Aldrich). Cell debris was removed by centrifugation; then, lysates were precleared with preequilibrated protein-G Sepharose (Pharmacia). Immunoprecipitation of CD18 was performed with the monoclonal antibody IB4, followed by an overnight incubation with protein-G Sepharose. Washed immunoprecipitates were boiled in a nonreducing sample buffer, separated by SDS/PAGE under nonreducing conditions, and transferred to nitrocellulose. Biotinylated proteins were labeled with streptavidin-peroxidase and visualized by enhanced chemiluminescence (Pharmacia).

Leukocyte-Endothelial Adhesion Assay. After experimental treatment, U937 or freshly purified human mononuclear cells were incubated with 2′7′-bis(carboxyethyl)-5(6)-carboxyfluorescein pentaacetoxymethyl ester (BCECF-AM) (Calbiochem) (21). Hydrolyzation of this ester following internalization resulted in cells labeled with BCECF. Labeled cells were then washed in HBSS and added to activated monolayers of HMEC-1. Culture plates were centrifuged to effect uniform settling, and adhesion was allowed for 10 min at 37°C. Monolayers were washed with HBSS, and fluorescence intensity was measured. Adherent cell numbers were determined from standard curves generated by serial dilution of known numbers of labeled cells. All data were normalized by subtraction of the fluorescent intensity of samples collected from monolayers incubated in buffer alone. In subsets of experiments, U937 cells were preincubated with the transcriptional inhibitor 5,6-dichlorobenzimidazole riboside (DRB) (Sigma–Aldrich) (22). In other experiments, U937 or human mononuclear cells were preincubated with the anti-CD18 monoclonal antibody IB4 (ATCC) or

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Abbreviations: HIF-1, hypoxia-inducible factor 1; HMEC-1, human microvascular endothelial cell; DRB, 5,6-dichlorobenzimidazole riboside; BCECF-AM, 2′7′-bis(carboxyethyl)-5(6)-carboxyfluorescein pentaacetoxymethyl ester; HRE, hypoxia response element.

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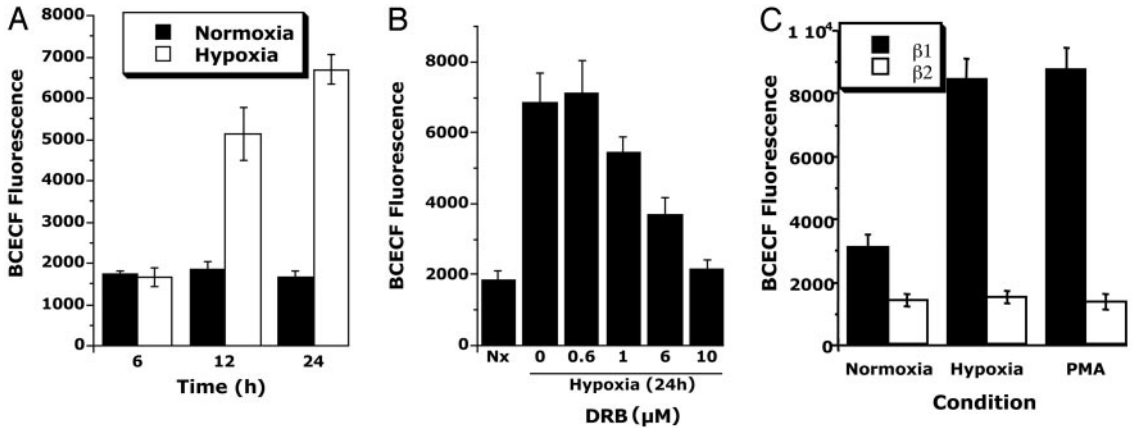

Fig. 1. Leukocyte hypoxia enhances β_2 integrin-dependent leukocyte adhesion to endothelia. (*A*) The myelocytic cell line U937 was exposed to normoxia [pO_2 147 torr (1 torr = 133 Pa), filled bars] or hypoxia (pO_2 20 torr, open bars) for the indicated periods of time. U937 cells were labeled with the fluorescent marker BCECF and assessed for adhesion to lipopolysaccharide-activated endothelia as described (21). (*B*) U937 cells were incubated with the transcription inhibitor DRB at the indicated concentrations during normoxia (Nx) or hypoxia (pO_2 20 torr, 24 h) and assessed for adhesion to activated endothelia. (*C*) U937 cells were exposed to normoxia (pO_2 147 torr) or hypoxia (pO_2 20 torr) and assessed for adhesion to activated endothelia in the presence or absence of inhibitory antibodies directed against β_1 or β_2 integrins. As a control, U937 cells were exposed to 10 ng/ml phorbol 12-myristate 13-acetate (PMA) for 12 h. This treatment is known to transcriptionally induce β_2 integrin expression (32, 33). All histograms represent the mean ± SE of three independent experiments.

the monoclonal antibody LM534 directed against β_1 integrins (Chemicon).

Flow Cytometry. Anticoagulated normal human blood was exposed to the indicated experimental conditions, and mononuclear cells were isolated by using density gradient centrifugation and Histopaque 1077 (Sigma–Aldrich). Cells were cooled to 4°C, blocked with media containing 10% bovine calf serum, and then incubated with the mouse antibody IB4 or control mouse IgG (Sigma–Aldrich). Cells were washed with cold HBSS followed by the addition of a fluorescein-conjugated goat anti-mouse secondary antibody (Cappel). Control cells were incubated with only the secondary antibody. The level of CD18 surface expression was determined by flow cytometry by using a FACScan flow cytometer (Becton Dickinson). Results were expressed as mean fluorescence intensity and analyzed by the Kolmogorov–Smirnov two-sample test.

Transcriptional Analysis. Messenger RNA was quantified by real-time PCR as described (23–25). Primer sets containing SYBR Green I (sense, antisense, and transcript size, respectively) were used for human CD18 (5′-CTG GTA GCA AAG CCC CCA CG-3′, 5′-TGG GTT TCA GCG AGG CTT GTG-3′, 325 bp), murine CD18 (5′-TGC GCC CCT CAC TGC TGC TTG-3′, 5′-GAG ATC CAT GAG GTA GTA CAG ATC-3′, 397 bp), and β-actin (5′-ACT GGA ACG GTG AAG GTG ACA G-3′, 5′-GGT GGC TTT TAG GAT GGC AAG-3′, 162 bp). (Molecular Probes). The analysis of β-actin was used to control for variations in the quantity of starting template.

Chromatin Immunoprecipitation Assay. U937 cells were fixed with 1% paraformaldehyde, and chromatin derived from isolated nuclei was sheared by using a F550 microtip cell sonicator (Fisher Scientific). After centrifugation, supernatants containing sheared chromatin were incubated with an anti-HIF-1α antibody (Novus Biologics, Littleton, CO) or control IgG. Protein A Sepharose was then added, the incubation was continued overnight, and immune complexes subsequently were eluted. Complexes were next treated with RNase and proteinase K and were extracted with phenol/chloroform and then with chloroform. DNA was precipitated, washed, dried, resuspended in water, and analyzed by PCR. The primers used in this analysis spanned 166 bp around the putative HIF-1-binding site within the CD18 promoter (sense, 5′-TGC AAC CCA CCA CTT CCT CCA-3′, and antisense, 5′-ACC CTC GGT GTG CTG GAG TC-3′).

CD18 Reporter Assays. U937 cells were transfected by electroporation with a mixture of the plasmid pRL-CMV that constitutively expresses *Renilla* luciferase (Promega) and either the plasmid CD18-WT or its parent pATLuc. CD18-WT contains the human CD18 gene promoter spanning nucleotides −79 to +19 cloned upstream of a promoterless firefly luciferase reporter gene whereas pATLuc lacks the CD18 promoter (16, 24, 26). After transfection, cells were subjected to hypoxia or normoxia and lysed, and luciferase activity was assessed by using a dual luciferase assay kit (Stratagene) and a Turner Designs (Sunnyvale, CA) luminometer. All firefly luciferase activity was normalized with respect to the constitutively expressed *Renilla* luciferase reporter gene. The functional importance of the HIF-1α-binding site 5′-ACGTG-3′ within the CD18-WT plasmid was assessed by its mutation to 5′-ACGAA-3′, 5′-ACAAA-3′, or 5′-AAAAA-3′ (27). The plasmids harboring these mutations were named CD18-HΔ2, CD18-HΔ3, and CD18-HΔ5, respectively. All mutations were confirmed by DNA sequencing. HIF-1α depletion was accomplished by using phosphorothioate derivatives of antisense (5′-GCC GGC GCC CTC CAT-3′) or control sense (5′-ATG GAG GGC GCC GGC-3′) oligonucleotides (23). Overexpression of HIF-1α was accomplished by transient transfection of GFP-HIF-1α. This construct was generated by cloning full-length HIF-1α (a kind gift from H. F. Bunn, Harvard Medical School, Boston) into pEGFP (Clontech). The influence of HIF-1α on CD18 promoter activity was assessed by cotransfecting GFP-HIF-1α with CD18-WT. The nuclear localization of GFP-HIF-1α was confirmed by immunofluorescence (28).

Results

Leukocyte Hypoxia Induces Increased Adhesion to Endothelia. Much is known about the influence of hypoxia on endothelial and epithelial function (29). In contrast, the direct impact of hypoxia on leukocyte function is almost completely unexplored (30, 31). Therefore, to begin to define this impact, we exposed the promonocytic cell line U937 to hypoxia and determined whether this hypoxia might influence its adhesion to activated endothelial cells. After 24 h of hypoxia, U937 cells exhibited almost a 5-fold increase in firm adhesion to activated endothelial cells (Fig. 1*A*).

We next determined whether hypoxia-induced leukocyte adhesion results from increased transcriptional activity. U937 cells were

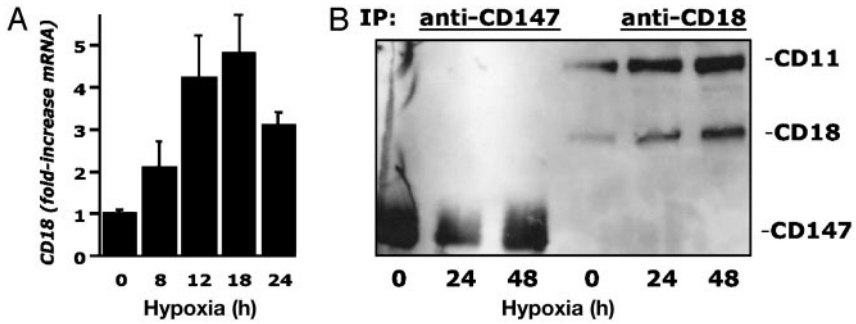

Fig. 2. Hypoxia induces leukocyte CD18 expression. (*A*) U937 cells were exposed to hypoxia (pO_2 20 torr) for the indicated periods of time. The levels of CD18 mRNA were then determined by real-time PCR relative to β-actin. Histograms represent the mean ± SE of three independent experiments. (*B*) U937 cells were exposed to the indicated periods of hypoxia. Cells were washed, surface proteins were biotinylated, and cells were lysed. CD18 and control CD147 were independently immunoprecipitated and then resolved in parallel by SDS/PAGE. Resultant Western blots were probed with avidin peroxidase. As indicated, the CD18 antibody immunoprecipitated heterodimeric complexes of CD11 and CD18. A representative experiment of three is shown.

subjected to hypoxia in the presence or absence of various concentrations of the transcriptional inhibitor DRB and assessed for changes in adhesion to activated endothelial cells (Fig. 1*B*). Preincubation of U937 cells with DRB inhibited hypoxia-induced adhesion in a concentration-dependent manner. These findings were not the result of cellular toxicity because lactate dehydrogenase release indicated that both in the presence and absence of DRB there was <10% cell death. Consequently, increases in U937 adhesion induced by hypoxia require new mRNA synthesis.

Firm adhesion of leukocytes to activated endothelia is known to depend on β_2 integrins (CD11/18). Therefore, we determined whether the component of hypoxia-induced leukocyte adhesion depended upon CD11/18. As shown in Fig. 1*C*, the component of increased leukocyte adhesion attributable to hypoxia was nearly completely inhibited by an anti-β_2 integrin antibody, but unaffected by an antibody directed against β_1 integrin. As a control, U937 cells were exposed to phorbol 12-myristate 13-acetate (PMA). This treatment resulted in the expected induction of β_2 integrin-dependent adhesion (32, 33).

In total, the results presented in Fig. 1 indicate that leukocyte hypoxia enhances β_2 integrin-dependent adhesion to activated endothelial cells. Furthermore, these results indicate that transcriptional mechanisms contribute to this enhanced adhesion.

Hypoxia Induces Leukocyte CD18 mRNA and Surface Protein Expression. Given the finding of a transcription-dependent increase in adhesion involving β_2 integrins, we next determined whether β_2 integrin expression was influenced by preexposure to hypoxia. Initially, we repeated the experiment shown in Fig. 1*A*, but this time examined CD18 mRNA levels by real-time PCR. As can be seen in Fig. 2*A*, CD18 mRNA is induced by almost 5-fold during hypoxia. Subsequently, we determined whether this increased mRNA expression translates into increased surface protein. U937 cells were subjected to 24 or 48 h of hypoxia, surface proteins were labeled with biotin, and CD18 was immunoprecipitated from lysates. Immunoprecipitates were resolved by SDS/PAGE, and Western blots were probed with avidin-peroxidase. As can be seen in Fig. 2*B*, this protocol precipitates CD11/18 heterodimeric complexes. The common CD18 subunit is readily apparent along with CD11 subunits that remain to be identified. These results indicate that the α and β subunits of the integrin heterodimer are induced in parallel by hypoxia. After 48 h, this induction reaches ≈6-fold for each subunit.

Hypoxia-Inducible CD18 Expression and Function *in Vivo* and *ex Vivo*. In the next phase of analysis we extended our study to models more physiologically relevant than the leukemia cell line U937. As depicted in Fig. 3*A*, real-time PCR analysis of leukocyte RNA derived from mice exposed to 6 h of hypoxia (18, 23, 25) revealed an induction of CD18 mRNA of ≈3-fold. In addition, after 4 h of hypoxia, over a 4-fold induction in CD18 mRNA was observed in total buffy coat leukocytes isolated from anticoagulated normal human blood (Fig. 3*B*). In a subsequent experiment, anticoagulated normal human blood was exposed to hypoxia for 24 h, and mononuclear cells were isolated and assessed for CD18 expression by flow cytometry (Fig. 3*C*). These studies revealed a >5-fold increase in CD18 mean fluorescence intensity. Finally, anticoagulated normal human blood was exposed to hypoxia for 24 h, and mononuclear cells were isolated, labeled with BCECF, and assessed for β_2 integrin-dependent adhesion to activated endothelial cells. As shown in Fig. 3*D*, human mononuclear cells subjected to hypoxia showed almost a 3-fold increase in adhesion. The component of increased mononuclear cell adhesion attributable to hypoxia was completely inhibited by an anti-β_2 integrin antibody but unaffected by an antibody that recognizes β_1 integrin. Taken together, the results depicted in Fig. 3 demonstrate that induction of CD18 expression and function by hypoxia extends beyond cultured leukocyte cell lines to both an *in vivo* murine model of hypoxia and to human whole blood *ex vivo*.

Role of HIF-1 in CD18 Induction by Hypoxia. In an attempt to gain specific insight into the mechanisms of CD18 induction by hypoxia, we began examining potential induction pathways used by other hypoxia response genes. Recently, HIF-1 has been demonstrated to be a dominant effector of changes in transcription in response to hypoxia (11). In the course of our experiments, we identified a potential HIF-1-binding site in the human CD18 gene promoter. This site contains the HIF-1 core sequence 5′-ACGTG-3′ between nucleotides +1 and +5 and the HIF-1 ancillary sequence 5′-CAGAC-3′ beginning 9 bp downstream (Fig. 4*A*) (34). We termed the region containing this core and ancillary sequence the hypoxia response element (HRE). Initially, we determined whether HIF-1 binds to this region of the CD18 promoter in U937 cells subjected to conditions of hypoxia. For these purposes, we used chromatin immunoprecipitation (ChIP) to analyze HIF-1α binding in live cells. As shown in Fig. 4*B*, ChIP analysis of nuclei derived from U937 cells revealed a dominant band of 166 bp in hypoxic, but not normoxic, samples. No bands were evident in control IgG immunoprecipitates, and preimmunoprecipitation samples revealed the predictable 166-bp band under conditions of both hypoxia and normoxia. Such results indicate that HIF-1α binds to the proximal 166-bp CD18 promoter in a region that bears a classic HRE. In addition, our results demonstrate that this binding is induced by hypoxia.

β_2 Integrin Gene Promoter Analysis. Three approaches were used to determine whether hypoxia might directly regulate β_2 integrin

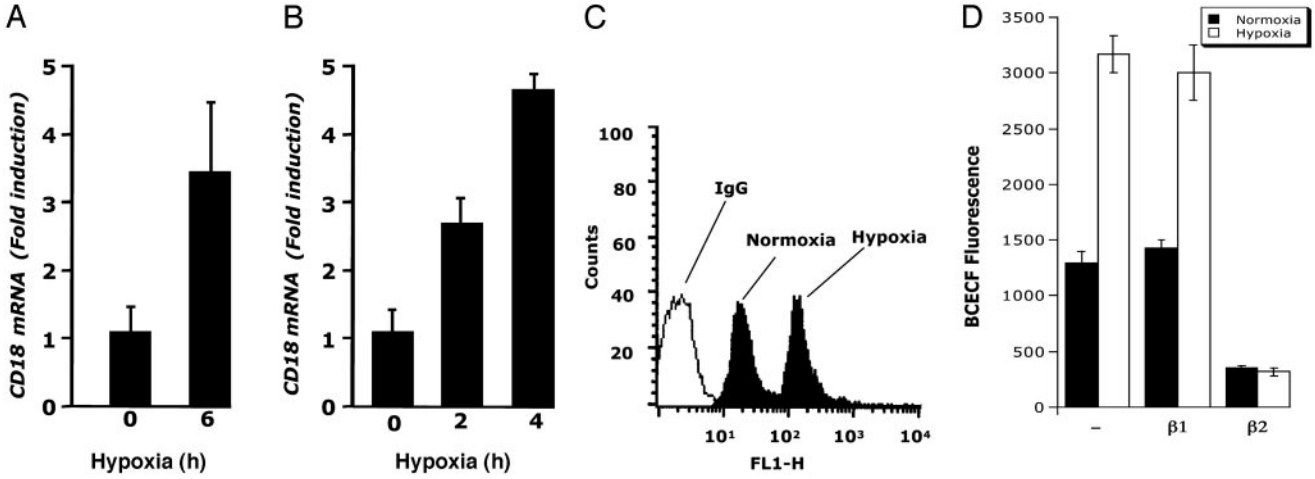

Fig. 3. Expression and function of hypoxia-inducible human and murine leukocyte CD18 *in vivo and ex vivo*. (*A*) For *in vivo* analysis, three WT BL6/129 mice were placed in chambers and allowed to breathe for 6 h in room air (21% O_2) or in a hypoxic atmosphere (8% O_2, 92% N_2). Animals were killed and exsanguinated, and whole leukocytes were isolated from buffy coats. The levels of CD18 mRNA were determined by real-time PCR relative to β-actin and are presented as fold-increases over normoxia. (*B*) For *ex vivo* analysis, anticoagulated whole human venous blood was exposed to the indicated periods of hypoxia (pO_2 20 torr). Whole leukocyte populations were isolated from the buffy coats, and the levels of CD18 mRNA were determined by real-time PCR relative to β-actin. These levels are presented as fold-increases over normoxia. (*C*) Flow cytometric analysis of CD18 expression on mononuclear cells after a 24-h incubation of anticoagulated whole human venous blood in normoxia or hypoxia. Mouse IgG is shown as a control. (*D*) Influence of anti-β_1 and -β_2 integrin antibodies on mononuclear cell adhesion to activated HMEC-1 after a 24-h incubation of anticoagulated whole human venous blood in normoxia or hypoxia. Control experiments with no antibody are also shown. All histograms represent the mean ± SE of three independent experiments.

promoter activity. First, a luciferase reporter construct containing the CD18 promoter assessed hypoxia inducibility. As shown in Fig. 5*A*, cells transiently transfected with the WT CD18 promoter showed, on average, a 23-fold increase in luciferase activity over normoxia controls after 24 h of hypoxia. Progressively severe mutation of the HIF-1α core sequence 5′-ACGTG-3′ resulted in a concomitant progressive decrease in promoter activity under hypoxic conditions. The most severe mutation of the core sequence caused, on average, a 91% decrease in activity. These findings indicate that the central HIF-1α-binding site is important for the CD18 hypoxia response. As a second approach, we determined the relative influence of HIF-1α expression on hypoxia-inducible β_2 integrin promoter activity. To do this, an antisense oligonucleotide directed against HIF-1α was used to block HIF-1α expression (23, 27). The influence of this oligonucleotide on CD18 promoter activity during hypoxia was then assessed (Fig. 5*B*). The directed loss of HIF-1α resulted, on average, in a 73% decrease in CD18 hypoxia inducibility. In a final assay, we examined the influence of HIF-1α overexpression on CD18 promoter activity during hypoxia. Here, we used the plasmid GFP-HIF-1α that expresses HIF-1α tagged with GFP. As shown in Fig. 6*A*, transient transfection of U937 cells with GFP-HIF-1α resulted in readily detectable nuclear GFP only in cells exposed to hypoxia. Using hypoxic conditions, we examined CD18 promoter activity when cotransfected with GFP-HIF-1α. As shown in Fig. 6*B*, overexpression of HIF-1α tagged with GFP caused, on average, 3.3- and 3.9-fold increases in promoter activity, respectively, when compared with a plasmid expressing GFP alone or effector plasmid absence. In contrast, overexpression of GFP-HIF-1α did not significantly influence CD18 promoter activity under normoxic conditions. Together, these data indicate that the induction of CD18 promoter activity in response to hypoxia is transcriptionally dependent upon HIF-1α.

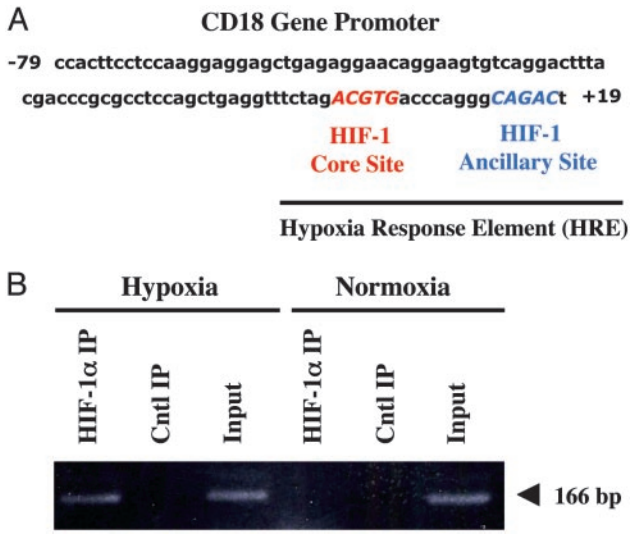

Fig. 4. Identification of an HRE in the CD18 promoter. (*A*) Depicted is the HRE within the CD18 gene promoter. The core motif 5′-ACGTG-3′ spans nucleotides +1 to +5, and the ancillary sequence 5′-CAGAC-3′ spans nucleotides +14 to +18. (*B*) Chromatin immunoprecipitation was used to examine HIF-1α binding to the CD18 promoter in normoxic and hypoxic U937 cells. Reaction controls included immunoprecipitations performed by using a nonspecific IgG monoclonal antibody (Cntl IP) and PCR performed by using whole leukocyte genomic DNA (Input). A representative example of three independent experiments is shown.

Discussion

Inflammation is characterized by localized hypoxia caused by increased metabolic demand (1–3). It has become apparent in recent years that the heterodimeric transcription factor HIF-1 is key to the molecular mechanisms by which hypoxia influences changes in cellular physiology. Genes induced by HIF-1 include those necessary for cellular, whole-tissue, and whole-animal adaptive responses to hypoxia (35, 36). Proteins induced by HIF-1 include a large number of enzymes involved in anaerobic metabolism, proteins (such as erythropoietin) important for erythrocyte mobilization, angiogenic peptides (such as vascular endothelial growth factor), and enzymes (such as inducible nitric oxide synthase) important for vasodilatory responses (37). Only recently has it been appreciated that HIF-1 may control gene expression in more diverse settings, such as inflammation (38, 39). The studies detailed

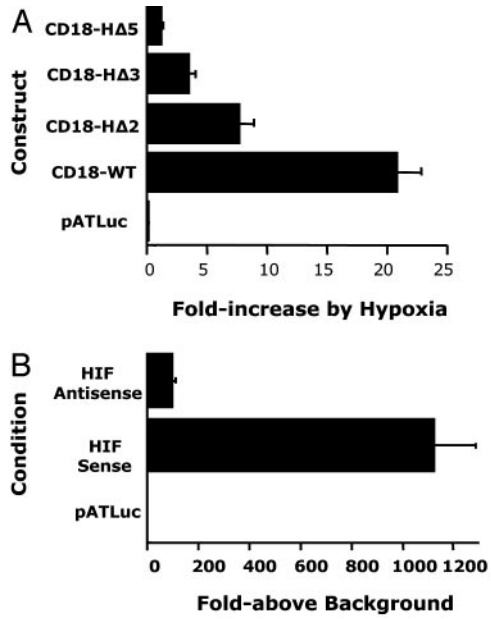

Fig. 5. Induction of the CD18 gene promoter by hypoxia. (*A*) U937 cells were transfected with the firefly luciferase construct CD18-WT, which contains the WT CD18 promoter, or with the mutant plasmids CD18-HΔ2, CD18-HΔ3, or CD18-HΔ5, which contain progressive substitutions within the HIF-1-binding site. Parallel transfections were performed by using the plasmid pATLuc, which represents the parent of CD18-WT and is empty of CD18 gene sequences. All transfection reactions contained the *Renilla* luciferase plasmid pRL-CMV. Transfected cells were exposed for 24 h to normoxia or hypoxia, and changes in the activity of the *Renilla* luciferase plasmid pRL-CMV were taken as reflecting variations in transfection efficiency. After correction for these variations, the level of firefly luciferase activity directed by the CD18 constructs above that conferred by the empty vector pATLuc under parallel conditions was determined. The fold increase in expression caused by hypoxia was then calculated for each firefly construct. (*B*) U937 cells were loaded with HIF-1α sense oligonucleotides or HIF-1α antisense oligonucleotides and transfected with CD18-WT or pATLuc. Cells were then exposed to hypoxia for 24 h, and luciferase activity was determined. After correction for variations in *Renilla* luciferase expression, the level of firefly luciferase activity directed by CD18-WT above that conferred by pATLuc under parallel conditions was determined. All histograms represent the mean ± SE of three independent experiments.

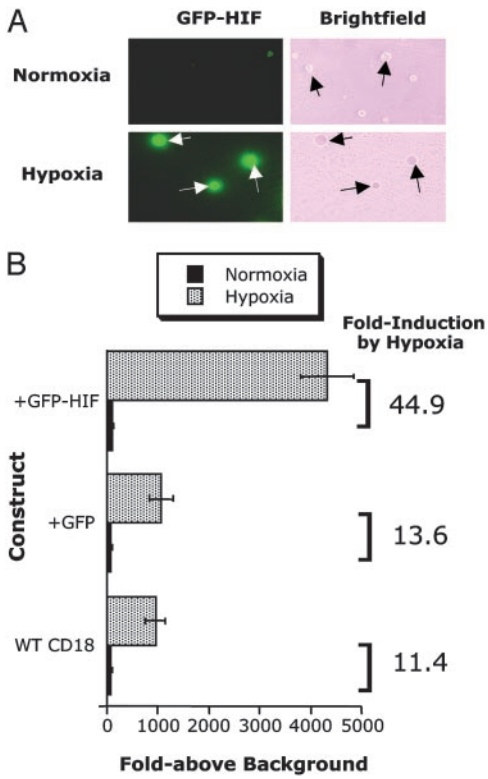

Fig. 6. Influence of HIF-1α overexpression on CD18 promoter activity. (*A*) U937 cells were transiently transfected with GFP-HIF-1α and exposed for 4 h to normoxia or hypoxia. Cells were washed, fixed with 1% paraformaldehyde, and mounted on glass coverslips. (*Left*) Cells were then imaged for nuclear GFP expression by immunofluorescence. (*Right*, arrows) Brightfield microscopy of the same field is depicted so the cells can be localized. A representative experiment of three is shown. (*B*) U937 cells were transiently transfected with CD18-WT alone (WT CD18), CD18-WT mixed with GFP-HIF-1α (+GFP-HIF), or CD18-WT mixed with the GFP vector (+GFP). Cells were then exposed to hypoxia or normoxia for 24 h, and the level of firefly luciferase activity directed by CD18-WT above that conferred by pATLuc under parallel conditions was determined. CD18 promoter activity is presented relative to the pATLuc background after correction for variations in *Renilla* luciferase expression. Histograms represent the mean ± SE of three independent experiments.

here reveal that, as a transcriptional regulator of β_2 integrin expression, HIF-1 may function to control the migration of myeloid leukocytes to inflammatory lesions.

HIF-1 expression depends predominantly on the degradation of its α-subunit in normoxia through the ubiquitin-proteasomal pathway (40, 41). The initiating step in this process is iron-dependent proline hydroxylation within the oxygen-dependent degradation domain (42, 43). Optimized hydrogen bonding to the hydroxyprolyl group then mediates the formation of a ubiquitin-E3 ligase complex constituted by the von Hippel Lindau (VHL) gene product, the elongins C and B, and Cullin 2 (40, 41). In contrast, hypoxic stabilization of HIF-1α initiates a multistep pathway of activation that includes hypoxia-dependent nuclear translocation and dimerization with the β-subunit. The assembled heterodimer is then able to bind HREs within target promoters (12). Most of the work on HIF-1 has focused on understanding the basic molecular mechanisms by which HIF-1 acts as a key mediator of cellular hypoxic responses and on its role in carcinogenesis (11, 44). However, recent studies have revealed a potentially central role for HIF-1 in endogenous protective pathways within a variety of inflammatory diseases, including respiratory distress syndrome, retinitis, diabetes, and arthritis (11). In addition, a recent cDNA profiling study suggested that HIF-1α expression is induced in ulcerative colitis (45).

It is now appreciated that leukocyte responses to hypoxia contribute to disease progression. In both acute conditions, such as ischemia-reperfusion injury, and chronic inflammation, such as occurs in arthritis, it is well documented that leukocytes are exposed to hypoxia (4). In addition, there is evidence to suggest that hypoxia may result in the increased expression of molecules, like the β_2 integrins, that mediate leukocyte adhesion and effect leukocyte extravasation, chemotaxis, and phagocytosis (46, 47). Little is known about the molecular mechanisms by which hypoxia causes such changes in leukocytes. However, Cramer *et al.* (48) have shown recently that HIF-1α expressed in myeloid cells is critical for cell-mediated inflammation. In these studies, the lysozyme M promoter was used to drive myeloid-specific expression of *Cre* recombinase, resulting in the generation of mice harboring conditional HIF-1α deletions within their myeloid lineage (48). Analysis of these mutant mice demonstrated that HIF-1α is critically important for successful inflammatory responses mediated by myeloid cells. The disruption of HIF-1α did not influence myeloid cell differentiation or development. However, HIF-1α deletion did result in significant metabolic defects manifest as profound impairment of myeloid cell motility, bacterial phagocytosis, and aggregation (48). Interestingly, these functional responses are dependent upon β_2 integrin expression (49).

Given that leukocytes seem to sense hypoxia and are key mediates of inflammation, we sought to determine whether hypoxia directly influences their physiology. The transition of leukocytes from being antiadhesive to proadhesive is characteristic of the inflammatory response. Our results show that this change in phenotype is influenced by leukocyte exposure to hypoxia. In addition, we show that increased adhesion is mediated by an induction of β_2 integrin expression. The changes in β_2 integrin expression most commonly thought to cause leukocyte adhesion occur within seconds and at the posttranslational level. Such changes include the mobilization to the cell surface of preformed intracellular pools of protein and activation of the affinity and avidity of surface protein for ligand (50–52). An important finding that we report here is that leukocyte adhesion induced by hypoxia and mediated by β_2 integrins depends on active transcription. Transcriptional mechanisms cause phenotypic changes on a time scale longer than posttranslational events. Therefore, we hypothesize that transcriptional induction of β_2 integrin expression is important for the maintenance of a sustained physiologic inflammatory response and likely contributes to the pathogenesis of conditions characterized by chronic inflammation.

In addition to CD18, our studies revealed that at least one of the α subunits of the β_2 integrin family is also induced by hypoxia. At present, we do not know the molecular mechanism responsible for this induction. Our sequence analysis of the regulatory regions of the four CD11 genes that encode the β_2 integrin α subunits revealed no apparent binding sites for HIF-1. The lack of obvious HRE sequences suggests that CD11 induction by hypoxia might be mediated by a different mechanism than that controlling expression of CD18. Nevertheless, it is compelling that coordinated induction of CD18 and CD11 expression does, in fact, occur. First, our experiments revealed that the level of CD18 and CD11 protein induction by hypoxia is nearly identical, suggesting the predictable stoichiometric 1:1 induction of surface protein. Second, the functional β_2 integrins exist only as heterodimeric complexes. Consequently, the formation of a functional integrin necessarily requires the parallel regulation of CD18 with at least one CD11 gene. Third, significant evidence exists that CD11 and CD18 are regulated in tandem (51). A number of transcription factors contribute to this regulation, including AP-1, MS-2, Purα, Sp1 and members of the Ets family (16, 33, 53–58). However, the molecular mechanisms that coordinate expression of the CD11 and CD18 genes during hypoxia remain to be determined.

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