Protein Kinase C β Is Required for Human Monocyte Chemotaxis to MCP-1*

Received for publication, April 22, 2003
Published, JBC Papers in Press, April 30, 2003, DOI 10.1074/jbc.M304182200

Kevin A. Carnevale and Martha K. Cathcart†
From the Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195

Monocyte chemotactic protein 1 (MCP-1) is important in attracting monocytes to sites of inflammation. Using predominantly pharmacological approaches, prior studies have indicated that serine/threonine kinases are involved in the MCP-1-induced signaling pathways. We report here that there is substantial inhibition of MCP-1-stimulated chemotaxis of human monocytes treated with inhibitors selective for the subset of serine/threonine kinases, protein kinase C (PKC). Selective inhibitors of PKC such as GF109203X and Calphostin C both caused ~80% inhibition of chemotaxis. Because these pharmacological inhibitors do not specifically inhibit individual PKC isoforms, we chose to use antisense oligodeoxyribonucleotides (ODN) to specifically reduce PKC isoform expression, first by inhibiting expression of the conventional PKC family, and next by using specific antisense ODN for PKCα and PKCβ. Conventional PKC-antisense ODN treatment completely and significantly inhibited monocyte chemotaxis to MCP-1, whereas sense-control ODN caused no significant inhibition. PKCβ-antisense ODN caused 89.2% inhibition of chemotaxis at its highest dose. In contrast, PKCβ-sense ODN and PKCα-antisense and -sense ODN were without effect. Further studies evaluating the calcium response that is triggered upon MCP-1 interaction with its receptor, CCR2, indicate that this response is not altered by antisense or sense ODN treatment, thus supporting our hypothesis that PKCβ is critical for post-receptor signal transduction downstream of the immediate calcium signal. These data contribute to our developing understanding of the signal transduction pathways involved in the chemotactic response of human monocytes to MCP-1 and uniquely identify the requirement for the PKCβ isoform in this important process.

Recruitment of monocytes into the peripheral tissue is essential for effector responses in inflammation and contributes significantly to the pathogenesis of atherosclerosis (1). The monocyte chemotactic response is usually a multistep process involving adhesion molecules, binding, rolling, flattening, and diapedesis. When cells move toward a concentration gradient of a chemotactic factor, the process is known as chemotaxis. Multiple proteins and lipids have been identified as having chemotactic activity; however, a family of small peptides known as chemokines is primarily responsible (2).

Chemokines are an important group of low molecular weight cytokines that play a major role in chemoattraction and activation of blood leukocytes. They are involved in multiple disease processes and are of major importance during acute and chronic inflammation (3). Chemokines are divided into four families depending on their location of the first two cysteines located nearest the N-terminus. The families include α, β, lymphotoxin, and fractalkine. α chemokines have one amino acid separating the first two cysteines (CXC). β chemokine cysteines are adjacent to each other (CC). In contrast, lymphotoxin has only one cysteine (C) in this region, and fractalkine is a membrane-bound protein that has three amino acids separating the first two cysteines (CXXXC) (2).

MCP-1* is a member of the CC or β chemokine family. It is expressed in vascular endothelial cells, vascular smooth muscle cells, monocytes, fibroblasts, and several cancer cell lines (4–11). MCP-1 can be induced by a variety of mediators including platelet-derived growth factor, interleukins IL-1 and IL-4, tissue necrosis factor α, vascular endothelial growth factor, bacterial lipopolysaccharide, and interferon γ (12–16). It exhibits its most potent chemotactic activity toward monocytes. MCP-1 binds to CCR2 and CCR11 receptors; however, binding to CCR11 does not result in increased intracellular calcium mobilization, which is essential for chemotaxis (17). Further, MCP-1 has a lower affinity for CCR11 than other chemokines. Our studies focus on the intracellular signaling pathways that are involved in the monocyte chemotactic response to MCP-1.

CCR2 is one of 11 β-chemokine receptors characterized by seven transmembrane domains and coupled to a GTP-binding protein (18). When MCP-1 binds to CCR2, it results in increased arachidonic acid release and influx of extracellular calcium, which is inhibited by Bordetella pertussis toxin treatments (19–21). Serine/threonine kinases are thought to be involved in post-CCR2 signaling as a result of numerous studies using pharmacological inhibitors (18, 22–24). General serine/threonine inhibitors such as C1, staurosporine, and H7 have all been shown to inhibit MCP-1-induced chemotaxis in treated monocytes (22). MCP-1 has been shown to induce a rapid and transient activation of mitogen-activated protein kinase in human monocytes, which was shown to be sensitive to H7 (24). This evidence indicates that serine/threonine kinases are required for MCP-1-stimulated chemotaxis. Because each of the aforementioned inhibitors inhibits PKC as well as

* This research was funded by National Institutes of Health Grant HL61971 (to M. K. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Cell Biology/NC10, Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195. Tel: 216-444-5222; Fax: 216-444-9429; E-mail: cathcam@ccf.org.

** The abbreviations used are: MCP-1, monocyte chemoattractant protein 1; PKC, protein kinase C; cPKC, conventional PKC; nPKC, novel PKC; ODN, oligodeoxyribonucleotides; PBS, phosphate-buffered saline; BCS, bovine calf serum; DMEM, Dulbecco’s modified Eagle’s medium; FMLP, formylmethionylleucylphenylalanine; PL, phosphatidylinositol; IL, interleukin; iPLA2, calcium-independent phospholipase A2; cPLA2, cytosolic phospholipase A2;
other serine/threonine kinases, we designed experiments to examine the role of PKC in regulating monocyte chemotaxis to MCP-1.

To date, at least 12 isoforms of PKC have been identified. They differ in cellular distribution, substrate specificity, and responsiveness to different activation. They are divided into three major groups: conventional PKCs (α, βI, βII, and γ); novel PKCs (δ, ε, η, and θ); and atypical PKCs (ζ, λ, μ, and η). Only cPKCs are calcium-dependent; however, all three groups are believed to participate in signal transduction (25).

In this study we first used general and selective pharmacological and peptide inhibitors of PKC to verify an involvement of PKC and to suggest the particular group of PKCs that might be regulating this process. We then tested antisense ODN to an mRNA sequence conserved among the cPKC group of PKCs and found that cPKC expression was required for the monocyte chemotactic response to MCP-1. Finally we utilized antisense ODN with the two cPKC enzymes that are expressed in monocytes, PKCα and PKCβ, and found that PKCβ plays a critical role in human monocyte chemotaxis to MCP-1.

EXPERIMENTAL PROCEDURES

Materials—H7, HA1004, GF109203X, and Calphostin C were purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA). Each of these reagents was dissolved in dimethyl sulfoxide as 100-fold stock solutions and stored at -20 °C prior to use. PKCβ C2-4 peptide inhibitor from BIOMOL Research Laboratories was dissolved in sterile water at 1 mM stock solution and stored at room temperature. Analysis was performed on a BD-LSR (BD Biosciences). The UV excitation (325 nm) was provided by a helium-cadmium laser; blue emission was detected at 510 nm. The results were analyzed using a TRANS-BLOT SD electrophoretic transfer cell (Bio-Rad). The membrane was blocked in 5% nonfat milk in PBS and 0.1% Tween 20 overnight at 4 °C and then probed with primary antibody. PKCα protein was detected with a 1:1000 dilution of anti-human recombinant PKCα (H-7) mouse monoclonal IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA) followed by incubation with anti-mouse IgG horseradish peroxidase (1:1000 dilution) (Transduction Laboratories, Lexington, KY). PKCβ protein was detected with a 1:1000 dilution of anti-human recombinant PKCβ (E-3) rabbit polyclonal IgG antibody for PKCβ protein or anti-human recombinant PKCβII (F-7) mouse monoclonal IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA). These were followed by incubation with 1:1000 dilution of anti-mouse IgG horseradish peroxidase (Transduction Laboratories). These antibodies recognized a band at the predicted migration of 85 kDa. The hybridization signals were detected using enhanced chemiluminescence detection reagents (Pierce) according to the manufacturer's guide and were followed by autoradiography.

Chemotaxis Assay—Monocyte migration was evaluated using a microchamber technique (30). Human recombinant MCP-1 (50 ng/ml) in DMEM with 0.1% bovine serum albumin was added to the lower compartment of the disposable 96-well chemotaxis chamber (NeuroProbe, Research Triangle Park, NC) in a volume of 20 μl. Migrated monocytes were counted in five high-power fields (×400) using a light microscope. All samples were tested in triplicate, and the data are expressed as the mean ± S.D.

Analysis of Intracellular Calcium Mobilization—This method has been described in detail previously by Robinovitch et al. (31). Briefly, cells were washed and resuspended at a final concentration of 3 × 10^6/ml of cell loading buffer (Hanks’ buffered salt solution with 1 mM calcium, 1 mM magnesium, and 0.5% bovine serum albumin). Probenecid (4 mM) was added in addition to 2 μg/ml acetoxyethyl ester of indo-1 (AM, Molecular Probes, Inc., Eugene, OR). Cells were loaded at 37 °C for 30 min, washed, resuspended in cell-loading buffer, and stored at room temperature. Analysis was performed on a BD-LSR (BD Biosciences). The excitation (325 nm) was provided by a helium/cadmium laser; blue emission was detected at 510–520 nm, and violet emission was detected at 400–440 nm. The results were analyzed using BD-GraphPad 4.2 software and plotted as percent of cells above threshold in violet/blue emission over time. Mean fluorescence in violet/blue emission over time was also determined.

RESULTS

H7, a general serine/threonine-kinase inhibitor including inhibition of PKC and cAMP-dependent protein kinase, suppressed MCP-1-stimulated chemotaxis of human monocytes in a dose-dependent manner causing 75% inhibition at the highest dose (Fig. 1A). HA1004, a chemical related to H7, inhibits cAMP-dependent protein kinase preferentially to PKC. HA1004 only caused partial inhibition of monocyte chemotaxis (about 40%), but this was not dose-dependent (Fig. 1B). The H7 finding confirms previously published data (23). We next investigated the effects of more selective inhibitors of PKC such as GF109203X and Calphostin C.

GF109203X is an inhibitor of the cPKC family, whereas Calphostin C inhibits PKC isoforms in both the cPKC family and the nPKC family (32). At 10 μM GF109203X inhibited MCP-1-induced chemotaxis of human monocytes by 80.6% (Fig. 1C). Calphostin C inhibited 79.1% at its highest dose (Fig. 1D). Inhibition mediated by both of these agents was dose-dependent. Calphostin C inhibition indicated that cPKC and/or nPKC isoforms were involved in regulating chemotaxis to MCP-1, but...
PKCβ Regulates Monocyte Chemotaxis to MCP-1

Since GF109203X caused equivalent inhibition, it narrowed our focus to the cPKC isoforms. It should be noted that trypan blue staining was performed at the end of all studies using pharmacological inhibitors, and no toxicity was observed.

To specifically test whether cPKC was playing a role in MCP-1-stimulated chemotaxis, we treated cells with a myristoylated peptide of PKCβII (amino acids 218–226) that was also conserved in PKCα, βi, and γ in the C2 domain (Biomol Research Laboratories). Among these isoforms, only PKCα and PKCβ are expressed in human monocytes. This resulted in dose-dependent, significant inhibition of chemotaxis (Fig. 2A). We also used a general cPKC-antisense ODN, which was directed to an mRNA sequence that was conserved among the cPKC members, PKCα, βi, and γ (29). Treatment with this antisense ODN totally inhibited the MCP-1-induced chemotaxis of human monocytes (Fig. 2B). There was very slight inhibition (9%) at 10 μM by the cPKC-sense ODN; however, this was not significant (Fig. 2B). There was no toxicity as assessed by trypan blue staining caused by peptide inhibitor, sense ODN, and antisense ODN at the highest doses.

To discriminate between regulation by PKCα and PKCβ, we used antisense ODN selective and specific to these PKC isoforms. These antisense ODNs were previously characterized and were shown to be selective for their targets in opsonized zymosan-activated human monocytes and not to affect the expression of other isoforms (28). We also characterized these antisense ODN in our system by looking at protein expression by Western blotting (Fig. 3). PKCα-antisense ODN specifically inhibited PKCα protein without affecting PKCβI- or PKCβII-protein levels (Fig. 3A). PKCα protein expression was inhibited by densitometry 80–90% after being normalized to β-tubulin expression over several experiments (data not shown). PKCβ-antisense ODN specifically inhibited PKCβI- and PKCβII-protein levels without affecting PKCα-protein levels (Fig. 3B). PKCβI-protein expression was inhibited by 70–95%, and PKCβII-protein expression was inhibited by 65–92%, both after being normalized to β-tubulin expression by densitometry over several experiments (data not shown).

We used these PKCα- and PKCβ-antisense ODNs to identify the cPKC isoform involved in MCP-1-stimulated chemotaxis of human monocytes. Antisense ODN for PKCβ inhibited the monocyte chemotactic response to MCP-1 by 75.4 and 89.2% at 5 and 10 μM, respectively (Fig. 4A). Although PKCβ-sense ODN showed some inhibition (23.8%) at the highest dose, this inhibition was not significant. No reduction of MCP-1-stimulated chemotaxis was seen in monocytes treated with PKCα-antisense or -sense ODN (Fig. 4B). Therefore, PKCβ expression is required for MCP-1-induced chemotaxis of human monocytes, whereas PKCα is not involved in this process.

Fig. 1. Pharmacological inhibitors of serine/threonine kinases and PKC suppress human monocyte chemotaxis. Monocytes were cultured in DMEM without serum and in the presence or absence of various concentrations of inhibitors for 1 h. Monocyte chemotaxis across a polycarbonate filter in response to MCP-1 (50 ng/ml) was then measured. All samples were tested in triplicate, and experiments were repeated three times. The results are expressed as the mean number of migrated monocytes in five high-power fields (5HPF) ± S.D. (400 × light microscope) and are from a representative experiment. Percentages of inhibition are shown in parentheses. *, p < 0.05; **, p < 0.005 from unpaired t tests. The squares represent the number of monocytes migrating in response to MCP-1, and the triangles represent the basal migration of monocytes in the absence of MCP-1.

Fig. 2. PKCβ C2–4 myristoylated peptide and cPKC-antisense ODN inhibit MCP-1-stimulated chemotaxis of human monocytes. Monocytes were cultured in DMEM without serum and in the presence or absence of various concentrations of the peptide inhibitor for 1 h. The squares represent the number of monocytes migrating in response to MCP-1, and the circle represents the basal migration of monocytes in the absence of MCP-1 (A). Monocytes were cultured in DMEM with 10% BCS and two different concentrations (5 and 10 μM) of cPKC-sense or -antisense ODN for 24 h (B). Monocyte chemotaxis to MCP-1 (50 ng/ml) was then measured. All samples were tested in triplicate. The results are expressed as the mean number of migrated monocytes ± S.D. in five high-power fields (400 × light microscope) from a representative experiment of two (A) and three (B) that were performed. Percentages of inhibition are shown in parentheses. *, p < 0.05; **, p < 0.005 from paired t tests.
PKCα and PKCβ antisense ODNs specifically inhibit their respective protein expression without affecting the other classical PKC isoform. Human monocytes were incubated with no ODN or 10 μM PKCα-sense ODN or 10 μM PKCα-antisense ODN at 37 °C for 24 h. 100 μg of total cytoplasmic protein was evaluated by Western blot for PKCα, PKCβI, and PKCβII protein (A). Monocytes were incubated with no ODN or 10 μM PKCβ-sense ODN or 10 μM PKCβ-antisense ODN at 37 °C for 24 h. 100 μg of total cytoplasmic protein was evaluated by Western blot for PKCβI, PKCβII, and PKCα protein (B).

To ensure that PKCβ was indeed functioning in a post-receptor signal transduction pathway, we evaluated the effect of the ODN on the MCP-1-induced calcium signal in human monocytes. The results of a representative experiment are shown in Fig. 5. Fig. 5A depicts the numbers of monocytes with increased calcium concentration in response to treatment with ionomycin. Fig. 5B shows similar results for monocytes responding to MCP-1. Neither sense ODN nor antisense ODN affected the number of responding cells (Fig. 5, C and D, respectively, as compared with B). We also evaluated the mean level of fluorescence in these same populations and found no effect of ODN treatment on mean violet over blue fluorescence. Treatment of monocytes with the PKCα-sense ODN and -antisense ODN also did not alter the calcium response induced by MCP-1 (data not shown).

**DISCUSSION**

Serine/threonine kinases have been implicated in regulating chemotactic responses to MCP-1 (18, 22, 23). Most studies have used general inhibitors with specificity for a wide range of kinases. More selective inhibitors have been used to investigate the contribution of mitogen-activated protein kinases, and although several investigators have speculated that PKC is required, this has not been definitively shown (23, 24, 33). In primary human monocytes, PKC has been shown to be part of the signaling pathways controlling the respiratory burst (29, 34, 35). PKC has been proposed to sustain calcium influx and be involved in the second phase diacylglycerol accumulation during chemotaxis (36, 37). PKC has also been shown to associate with cytoskeletal elements (38–42). To date, no specific inhibitors of PKC have been evaluated for their effects in regulating the chemotaxis of monocytes to MCP-1. Because we have previously shown that antisense ODN can specifically inhibit certain PKC isoforms, we used these tools to address the question of whether PKC participates in regulating MCP-1-induced chemotaxis and if so, what specific isoforms are involved.

PKC has been studied in many different cell types and has been shown to be important in chemotaxis to different stimuli. The chemotactic responses of cells such as lymphocytes, macrophages, fibroblasts, endothelial cells, and neutrophils have been reported to involve PKC to stimuli such as fMLP, serum...
amyloid A, macrophage-inflammatory protein α, secretinurin, platelet-activating factor, fibroblast growth factor, cytokine-induced neutrophil chemoattractant-1, and others (43–53). Although these studies suggest a general involvement of PKCs in chemotaxis, many of them used general or selective pharmacological inhibitors, which can possibly affect other pathways. Our experience with general pharmacological inhibitors corroborates previous studies and shows that serine/threonine kinases are indeed required for MCP-1-stimulated chemotaxis of human monocytes (Fig. 1). In addition, cAMP-dependent protein kinase may be partially involved because of the partial inhibition by H89, but the lack of dose-dependent inhibition does not strongly support a role for cAMP-dependent protein kinase (Fig. 1). Because we did not pursue the role of cAMP-dependent protein kinase with more selective inhibitors or antisense ODN, definition of its exact role in chemotaxis to MCP-1 will require further investigation.

A synthetic peptide, Trp-Lys-Tyr-Met-Val-d-Met, has been shown to be a chemoattractant stimulus to phagocytic leukocytes including human monocytes (54). Even though the peptide-induced monocyte chemotaxis is pertussis toxin-sensitive, the peptide-specific receptor is different from receptors for MCP-1 and is insensitive to GF109203X. Chemotaxis to Trp-Lys-Tyr-Met-Val-d-Met is sensitive to genistein (a tyrosine kinase pharmacological inhibitor) and a Rho-A inhibitor. This finding highlights the concept that different chemotactic stimuli for human monocytes can act through different receptors and involve different signaling pathways.

A study using pharmacological inhibitors indicated that phosphatidylinositol 3-kinase or extracellular signal-regulated kinase activity are not required for monocyte migration toward MCP-1, RANTES (regulated on activation, normal T cell expressed and secreted), MIP (macrophage inflammatory protein)-1α, or fMLP using wortmannin, LY294002, and PD098059 (43). However, other studies in which monocytes were pretreated with wortmannin and PD098059 showed direct inhibition of MCP-1-stimulated chemotaxis (24). Therefore, some studies using pharmacological inhibitors are conflicting. Other studies from knockout mice indicate the importance of phosphatidylinositol 3-kinase in macrophage chemotaxis to a variety of stimuli; however, chemotaxis to MCP-1 was not evaluated (55).

Additionally, the study investigating phosphatidylinositol 3-kinase and extracellular signal-regulated kinase focused on MCP-1-induced chemotaxis of monocytes and found that GF109203X did not affect monocyte migration alone; however, pretreatment of monocytes with phorbol 12-myristate 13-acetate significantly impaired the response to MCP-1 and other chemotactic agents such as fMLP (43). This study showed that phorbol 12-myristate 13-acetate inhibition was reversed by co-treatment with GF109203X implying that PKC activation is capable of desensitizing MCP-1- and fMLP-induced monocyte chemotaxis. That report is different from our results, which show that pretreatment with GF109203X directly inhibits MCP-1-stimulated chemotaxis of human monocytes (Fig. 1C). Their study relied entirely on pharmacological inhibitors/activators and did not address the particular isoforms of PKC involved (43). In addition to our observations that GF109203X inhibits monocyte chemotaxis to MCP-1, we also show that Calphostin C is an equally effective inhibitor of this process (Fig. 1D). Because there are conflicting studies using pharmacological inhibitors and the specificity of these drugs remains uncertain, we found it necessary to use other more selective and specific inhibitors such as antisense ODN.

One of our important findings is that a cPKC peptide inhibitor and cPKC-antisense ODN were able to substantially and significantly inhibit MCP-1-induced chemotaxis of human monocytes (Fig. 2). Because PKCy is not expressed in human monocytes, this led us to focus on PKCa and PKCβ isoforms. We show the isoenzyme specificity of PKCa- and PKCβ-antisense ODN in primary human monocytes (Fig. 3) and indicate that these reagents do not inhibit the initial receptor response such as intracellular calcium mobilization (Fig. 5). Our novel finding is that PKCβ is an essential cPKC isoform in MCP-1-stimulated chemotaxis of human monocytes in vitro (Fig. 4A). In contrast, PKCa-antisense ODN had no effect (Fig. 4B).

An atypical PKC isoform, PKCζ, was shown to be essential in the chemotaxis of neutrophils to IL-8 and fMLP (56). This study used pseudosubstrate peptides to PKCζ and PKCs and demonstrated that PKCζ, and not PKCζ, was essential in adhesion and chemotaxis to IL-8 and fMLP in neutrophils. Lastly, the study showed that pharmacological inhibitors to classic and novel PKC families such as Calphostin C, G06850, and G06976 had no effect on adhesion or chemotaxis in pretreated neutrophils. It is possible that α chemokines such as IL-8 activate different PKC isoforms than β chemokines like MCP-1. Thus, different cells appear to utilize alternative regulatory pathways in chemotaxis, and these differences may serve as a focus for further investigation.

We have previously shown that,iPLA2 is essential in human monocyte chemotaxis to MCP-1 using antisense ODN and have confirmed the importance of cPLA2 (57). Interestingly, iPLA2-antisense ODN inhibition of MCP-1-stimulated chemotaxis was restored by lysophosphatidic acid and not arachidonic acid, whereas the reverse was found for cPLA2-antisense ODN inhibition. The exact mechanism of their involvement in chemotaxis is unknown; however, they seem to be independently involved. Both novel and conventional PKC isoforms have been implicated in phosphorylation of iPLA2 in ventricular myocytes and in the zymosan-activated macrophage cell line, P388D1, cells (58, 59). The phosphorylation of these enzymes after MCP-1 stimulation remain unknown; however, this is an important direction for future studies.

In summary, relatively little is known about the regulation of monocyte chemotaxis to MCP-1. The majority of studies have used pharmacological inhibitors to understand possible pathways that may be involved. Some pathways that have been implicated as regulators of monocyte chemotaxis to MCP-1 include phosphatidylinositol 3-kinase, mitogen-activated protein kinases, protein kinase C, and PLA2 (18, 24, 57, 60, 61). Our study clearly demonstrates that the cPKC isoform, PKCβ, is essential for human monocyte chemotaxis to MCP-1. The exact role that PKCβ is playing in the signaling pathway is unknown and is the topic of our current studies. PKCβII has previously been shown to bind and be activated by cytoskeletal elements such as F-actin (62). Additionally, T-cell initiation of crawling locomotion via integrin receptors is dependent on the phosphorylation of microtubules by PKCβII (63). Whatever mechanism PKCβ regulates, it may serve as a specific target for inhibiting human monocyte chemotaxis in different disease processes while possibly not affecting neutrophil migration or monocyte migration to other chemokines.

Acknowledgments—We thank Dr. Bela Anand-Apte for advice on the chemotaxis assay and Portia Payton for help in counting cells and compiling data from the chemotaxis assays.

REFERENCES
1. Ross, R. (1999) N. Engl. J. Med. 340, 115–126
2. Rollins, B. J. (1997) Blood 90, 909–928
3. Lauster, A. D. (1998) N. Engl. J. Med. 338, 436–445
4. Jiang, Y., Valente, A. J., Williamson, M. J., Zhang, L., and Graves, D. T. (1990) J. Biol. Chem. 265, 18312–18321
5. Valente, A. J., Graves, D. T., Vialle-Valentin, C. E., Delgado, R., and Schwartz, C. J. (1988) Biochemistry 27, 4162–4168
6. Rollins, B. J., Stier, P., Ernst, T., and Wong, G. G. (1989) Mol. Cell. Biol. 9,
