Research Article

Cell migration to the chemokine CXCL8: Paxillin is activated and regulates adhesion and cell motility

E. Cohen-Hillel, R. Mintz, T. Meshel, B.-Z. Garty and A. Ben-Baruch

Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978 (Israel), Fax: +972-3-6422046, e-mail: aditbb@tauex.tau.ac.il

Schneider Children's Medical Center, Pediatrics B, Petah Tikva, Tel Aviv University, Tel Aviv 69978 (Israel)

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Abstract. The chemokine CXCL8 is a powerful inducer of directional cell motility, primarily during inflammation. In this study, we found that CXCL8 stimulation led to paxillin phosphorylation in normal neutrophils, and that both CXCL8 receptors (CXCR1 and CXCR2) mediated CXCL8-induced paxillin phosphorylation. In CXCR2-transfected cells, the process depended on $G_{ia}$ and $G_{is}$ coupling to CXCR2. Dominant negative (DN) paxillin increased CXCL8-induced adhesion and migration, indicating that endogenous paxillin keeps migration at sub-maximal levels. Furthermore, using activating antibodies to $\beta 1$ integrins, analyses with focal adhesion kinase (FAK) DN variant (FRNK) and co-immunoprecipitations of FAK and paxillin, we found that $\beta 1$ integrin ligation cooperates with CXCL8-induced stimulation, leading to FAK activation and thereafter to FAK-mediated paxillin phosphorylation. Our findings indicate that paxillin keeps directional motility at a restrained magnitude, and suggest that perturbations in its activation may lead to chemotactic imbalance and to pathological conditions associated with excessive or reduced leukocyte migration.

Keywords. Cell migration, chemokines, CXCL8, CXCR2, paxillin.

Introduction

Cell migration is an essential process in organ homeostasis, inflammation and angiogenesis. Members of the chemokine superfamily are important regulators of leukocyte directional motility to inflammatory sites and to lymphoid organs [1–4]. Of the different chemokines, CXCL8 (Interleukin 8) is a prime inducer of neutrophil directed migration to acute inflammatory sites, and it was also found to promote the motility of other leukocyte sub-types [5–8]. However, the roles of CXCL8 in migration are not limited to leukocytes, as it was found to induce the motility of tumor cells and endothelial cells, thus being a potent angiogenic factor [9–12]. In the inflammatory context, CXCL8-induced migration is essential for the immune integrity of the host. However, since cell motility is a dynamic, sequential, and tightly regulated process, its inappropriate regulation may lead to pathological conditions. For example, intense CXCL8 activities may lead to exacerbated leukocyte migration, resulting in inflammatory diseases such as chronic obstructive pulmonary disease, acute respiratory distress syndrome and others. Alternatively, desensitization of CXCL8-induced migration may form part of the basis of
pathological conditions in which reduced response to inflammatory signals is observed [13–18]. In view of the important roles played by CXCL8 in inflammatory processes, we wished to provide insights into the regulation of CXCL8-induced directional motility, and to identify the molecular mechanisms controlling adhesion and migration in response to this chemokine. We have previously investigated the involvement of the protein tyrosine kinase focal adhesion kinase (FAK) in CXCL8-induced directed migration, mediated through the two high-affinity CXCL8 receptors, the G protein-coupled receptors CXCR1 and CXCR2 [6–8, 19, 20]. Our findings showed that FAK phosphorylation and activation are essential for CXCL8-induced migration [19]. Also, we discovered that fine-tuning of CXCR1- and CXCR2-mediated signals is important for regulation of migration, and that CXCR2 undergoes more complex regulation than CXCR1 [19, 20].

In the present study we further explored the regulation of CXCL8-induced directed cell motility by focusing on paxillin, a focal adhesion scaffold protein that binds many proteins regulating the actin cytoskeleton, cell adhesion and cell migration. Paxillin is stimulated by integrin ligation induced by adhesion to extracellular matrix (ECM) proteins, and by signals delivered by activated G protein-coupled receptors and growth factor receptors [21–25]. The activation of paxillin is mediated mainly by phosphorylation of four tyrosine residues located at its N-terminal domain. Thus far, three tyrosine kinases were found to bind paxillin and to induce its phosphorylation on tyrosine residues, including FAK, proline-rich tyrosine kinase 2 (Pyk2) and Src family members. Importantly, of the four tyrosine phosphorylation sites of paxillin, the Y118 and Y31 are those having major importance for regulation of cell migration [21, 22, 24, 26–28].

By controlling the organization and function of focal contact areas and of the cytoskeleton, paxillin controls processes of adhesion and motility. While many studies indicated that paxillin is a positive regulator of cell migration, others have suggested that paxillin activation retards cell motility. Recent studies suggested that the opposing effects of paxillin on cell migration may depend on the type of migration cues delivered to the cells, and that paxillin controls cellular migration differently in conditions of random motility as compared to directional migration [21–23, 29–33]. To date, many studies have addressed the activation of paxillin in response to integrin engagement with ECM proteins; however, in most cases the analyses were not performed in the presence of chemotactic ligands. Aspects regarding paxillin activation in the context of chemotactic signals are of major interest, because chemoattractants play key roles in integrin activation [1, 23, 34, 35], and because integrin stimulation is a predominant regulator of paxillin activation [21–25]. When paxillin regulation was investigated in the presence of chemokine signals, the majority of studies addressed the homeostatic chemokine CXCL12 (SDF-1), showing that it induces paxillin phosphorylation. However, only very few of these studies analyzed the direct roles played by paxillin in chemokine-induced directional migration, and the mechanisms regulating its activation [29, 36–40].

To better understand the involvement of paxillin in motility induced by chemotactic stimuli, we focused in this study on CXCL8, because of its prime activities in the inflammatory process. The findings obtained in our study indicate that, following CXCL8-induced activation, endogenous paxillin keeps migration and adhesion at sub-maximal levels; therefore, paxillin acts as a negative regulator of the directional motility induced by this chemokine. Furthermore, our findings indicate that CXCL8-induced paxillin phosphorylation depends on an initial step of integrin activation, and that this step cooperates with CXCL8-induced signals, leading to stimulation of FAK, and eventually to FAK-mediated paxillin phosphorylation and activation.

Overall, this study provides novel insights into the roles of paxillin in directional migration induced by an inflammatory chemokine, and indicate that paxillin acts as a negative regulator of motility in response to directional cues provided by CXCL8. Our observations indicate that paxillin is tightly controlled, and suggest that inappropriate regulation of paxillin upon CXCL8 stimulation may lead to chemotactic imbalance in pathological conditions.

Materials and methods

Transfected cells. Constructs expressing wild-type (WT) human CXCR1 and CXCR2 were generated, followed by full-length sequencing that verified the desired sequences. Stable polyclonal CXCR1 and/or CXCR2 transfectants of rat basophilic leukemia (RBL) 2H3 cells and of human embryonic kidney (HEK) 293 cells were established with these constructs. CXCR1 or CXCR2 were expressed by the transfected cells at similar levels in over 85% of transfected RBL and HEK cells. All cell types responded potently to CXCL8, as determined by migration and internalization assays ([19, 20, 41–54] and figures presented in the current paper). Untransfected and/or empty vector-transfected RBL and HEK cells did not express CXCL8 receptors and did not bind CXCL8 ([42–44, 52, 53] and data not shown).