

Editorial

Integrins as Co-receptors of Fractalkine

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Integrins are a family of cell adhesion receptors that recognize extracellular matrix (ECM) ligands, cell surface ligands and soluble ligands (e.g., growth factors [1,2]). Ligation of integrins triggers a large variety of signal transduction events such as proliferation, shape, motility, gene expression and differentiation. It has been well established that integrins are required for cytokine/growth factor signaling (integrin-cytokine crosstalk). Current models suggest that cytokines bind to cytokine receptors and integrins bind to ECM ligands, and the two separate signals merge inside the cells. Recent studies in our lab suggest that these models may need to be revised. We reported that several cytokines (fibroblast growth factor-1 (FGF1), insulin-like growth factor-1 (IGF1), neuregulin-1 and fractalkine (FKN/CX3CL1)) directly bind to both integrins and cytokine receptors simultaneously and induce ternary complex formation on the cell surface (integrin-cytokine-cytokine receptor) [3-12]. The integrin-binding defective cytokine mutants are not capable of signal transduction and ternary complex formation. Notably, we found that the mutants of FGF1, IGF1 and FKN act as antagonists of cytokine signaling (dominant-negative mutants) [4,5,10,12], suggesting that the integrin-binding defective cytokine mutants may have potential as therapeutics. In this editorial, we focus on the roles of integrins-FKN (chemokine) crosstalk in leukocytes based on our recent findings. We propose a new model of FKN signaling, in which integrins act as a co-receptor of FKN.

The recruitment of leukocytes from the circulation to inflammation sites is a critical event in the inflammatory response and includes several processes [13-17]. Leukocyte trafficking requires adhesion molecules (e.g., selectin, integrins), chemokines and cytoskeletal regulators [18-23]. FKN is a CX3C chemokine that plays a role in inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus [24-27]. Unlike most chemokines, FKN is expressed as a membrane-bound form on endothelial cells [28]. FKN is composed of 373 total amino acids consisting of an N-terminal chemokine domain (residues 1-76), a mucin-like stalk (residues 77-317), a transmembrane α helix (residues 318-336), and a short cytoplasmic tail (residues 337-373) [29]. FKN's specific receptor CX3CR1 (a G-protein coupled receptor) is expressed in

NK cells, T cells, monocytes, platelets, and vascular smooth muscle cells [30-33]. FKN binding to CX3CR1 mediates interaction between inflammatory cells (monocytes and T cells) and endothelial cells. The chemokine domain of FKN (FKN-CD) is presented at the top of the membrane-bound mucin-like stalk and FKN itself acts as an adhesion molecule. In addition to the intrinsic adhesion function, FKN enhances cell adhesion through integrin activation. Therefore, the engagement of both CX3CR1 and integrins through FKN and integrin ligands (e.g., ICAM-1 and VCAM-1) results in enhanced cell adhesion [29,34]. FKN-CX3CR1 interaction can increase integrin affinity through G-protein [22,35-41] and enhance cell adhesion to activated endothelial cells. Thus, it has been proposed that FKN-mediated adhesion does not involve the direct binding of integrins to FKN.

However, we discovered that FKN-CD directly binds to integrins ($\alpha\beta3$ and $\alpha4\beta1$) and induces ternary complex formation (integrin-FKN-CX3CR1) on the cell surface in leukocytes [12]. We also identified that Lys36 and Arg37 in FKN-CD are critical residues for the integrin binding. We generated an integrin-binding defective FKN mutant (K36E/R37E). The mutant still binds to CX3CR1 well, but the mutant is defective in integrin activation, cell adhesion and ternary complex formation in leukocytes. Thus, our studies suggest that FKN-CX3CR1 interaction is not sufficient for these functions. Based on these findings, we propose a novel model of FKN signaling, in which the direct integrin-FKN interaction and subsequent ternary complex formation (integrin-FKN-CX3CR1) are required for FKN function in leukocytes (e. g., integrin activation and cell adhesion).

Why direct integrin binding to the chemokine domain of FKN was overlooked? It is known that integrin-ligand interaction is dependent on divalent cations. Therefore, since EDTA does not block FKN binding to cell surface, it has been proposed that this interaction is integrin-independent [30]. However, we demonstrated that EDTA does not fully suppress FKN-CD binding to integrins $\alpha\beta3$ and $\alpha4\beta1$. We believe that EDTA is insufficient to inhibit FKN-integrin interaction since FKN-CD is an extremely high affinity ligand for integrins ($KD=3\times10^{-10}$ M). Also, previous studies used the chemokine domain (76 amino acid residues) fused with large secretory placenta alkaline phosphatase (484 amino acid residues) (CX3CL1-SEAP) [34,36]. We suspect that CX3CL1-SEAP is defective in integrin binding possibly due to steric hindrance. We used the chemokine domain with a relatively small tag (34 amino acid residues). Possibly, that is why we were able to detect integrin-FKN-CD interaction.

If ternary complex formation is required for FKN signaling, it is predicted that the mutant will exert as an antagonist of this signaling pathway. Consistent with the prediction, we demonstrated that excess K36E/R37E suppresses integrin activation *in vitro*, and leukocyte infiltration in thioglycollate-induced peritonitis *in vivo* [12]. These findings suggest that the mutant acts as a dominant-negative antagonist of CX3CR1. The K36E/R37E mutant may have

potential as a therapeutic agent in inflammatory diseases.

In this editorial, we discussed our recent findings in cytokine-integrin crosstalk, particularly the roles of integrins in FKN signaling. We propose a novel model of FKN signaling, in which the direct integrin-FKN interaction and subsequent ternary complex formation (integrin-FKN-CX3CR1) are required for FKN function. The integrin-binding defective FKN mutant is not only a useful tool for understanding the roles of integrins, but also has therapeutic potential as an anti-inflammatory agent.

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