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*J Immunol* 2007; 179:6305-6310; doi: 10.4049/jimmunol.179.9.6305

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HVEM Signaling in Monocytes Is Mediated by Intracellular Calcium Mobilization

Sook-Kyoung Heo,* Min-A Yoon,† Sang-Chul Lee,‡ Seong-A Ju,‡ Jang-Hyun Choi,§ Pann-Ghill Suh,§ Byoung S. Kwon,*§ and Byung-Sam Kim2*†‡

Herpes virus entry mediator (HVEM) is a member of the TNF receptor (TNFR) superfamily and is expressed on many immune cells, including T and B cells, NK cells, monocytes, and neutrophils. Interaction of HVEM with its ligand, LIGHT, costimulates T cells and increases the bactericidal activity of monocytes and neutrophils. The interaction recruits cytoplasmic TNFR-associated factor adaptor proteins to the intracellular domain of HVEM. This leads to NFκB activation as a result of IκBα degradation and/or JNK/AP-1 activation, and ultimately results in the expression of genes required for cell survival, cytokine production, or cell proliferation. In this study, we show that treatment of human monocytes with recombinant human LIGHT (rhLIGHT) induces rapid elevation of intracellular calcium concentration ([Ca2+]i) in a HVEM-specific manner in parallel with TNF-α production, and enhances the bactericidal activities of monocytes. Immunoprecipitation and Western blotting analyses revealed phosphorylation of phospholipase C-γ1 (PLC-γ1) but not PLC-γ2. rhLIGHT-induced Ca2+ response was completely abolished by silencing PLC-γ1, or preincubating monocytes with PLC inhibitors, antagonists of the inositol-1,4,5-triphosphate receptor, or [Ca2+]i chelators. Furthermore, these PLC/Ca2+ inhibitors also blocked rhLIGHT-mediated IκBα degradation, generation of reactive oxygen species, TNF-α production and the bactericidal activities of monocytes. Our results indicate that Ca2+ is a downstream mediator of the LIGHT/HVEM interaction in monocytes. The Journal of Immunology, 2007, 179: 6305–6310.

LIGHT (homologous to lymphotoxins, shows inducible expression, and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator (HVEM), a receptor expressed by T lymphocytes),3 a member of the TNF ligand superfamily, is predominantly expressed on immune cells such as T cells, monocytes, NK cells, and immature dendritic cells (1). LIGHT binds to two membrane receptors, HVEM and lymphotixin β receptor (LTβR) (2). HVEM is also expressed on immune cells, but LTβR is only expressed on stromal cells in the spleen (3). Therefore, LIGHT/HVEM interactions between immune cells are thought to regulate a variety of immune responses. For example, the LIGHT/HVEM interaction costimulates T cell proliferation and cytokine production (4), induces dendritic cell maturation (5), stimulates Ig production in B cells (6), and activates NK cells (7). Previously, we have also shown that this interaction contributes to the antibacterial activity of monocytes and neutrophils by enhancing phagocytosis, the production of ROS, NO, and proinflammatory cytokines, and bactericidal activity (8).

Upon binding of LIGHT, it has been shown adaptor proteins referred to as TNFR-associated factors (TRAFs) are recruited to the cytoplasmic domains of HVEM (9), which leads to NFκB activation (4). However, the relevance of this signaling pathway to immune responses mediated by HVEM has not been specifically demonstrated. Recently, we observed that inhibitors of NAD(P)H oxidase blocked HVEM-mediated activation of monocytes and neutrophils (8). Because the activity of NAD(P)H oxidase can be regulated by the intracellular calcium ion concentration ([Ca2+]i), we speculated that HVEM signaling in monocytes might involve Ca2+. Monocytes have the highest level of HVEM of any immune cell (8). They play critical roles in clearing bacteria from infection sites and contribute to a wide range of immune and homeostatic functions in which Ca2+ is a major signal mediator.

In this study, we report that in human monocytes, HVEM stimulation by rhLIGHT induces rapid [Ca2+]i, rise in parallel with tyrosine phosphorylation of phospholipase C-γ1 (PLC-γ1). Silencing PLC-γ1 or HVEM by small interfering RNA (siRNA) abrogated rhLIGHT-induced calcium responses and incubation of cells with PLC/[Ca2+]i inhibitors such as, U73122, genistein, xestospongin C (XeC) and BAPTA-AM abolished recombinant human LIGHT (rhLIGHT)-induced NFκB activation, production of TNF-α and ROS, and bactericidal activity, as well as the Ca2+ responses of monocytes.

Materials and Methods

Reagents

rhLIGHT was purchased from R & D Systems, and diluted in 0.1% BSA-PBS buffer. According to the manufacturer, the rhLIGHT contains <0.1 ng of endotoxin contamination per μg of protein, as determined by the low density lipoprotein method. The antihuman HVEM mAb (clone 108, ID4,
4G2, 3G06 were developed in this laboratory (8, 10). Herpes simplex virus-1 glycoprotein D (HSV-1gD) was purchased from Advanced Biotechnologies. Anti-PLCγ1, anti-PLCγ2, and anti-phosphorytrosine mAb were provided by Dr. S. H. Ryu (11) (Pohang University, Pohang, South Korea). All other chemicals were purchased from Sigma-Aldrich. Heat-inactivated (HI) Abs and HI-rhLIGHT were prepared by incubation at 80°C for 20 min, and neutralized rhLIGHT was prepared by incubating rhLIGHT with anti-LIGHT mAb (1 μg/ml) at 0°C for 30 min.

Preparation of human monocytes and cell lines
Mononuclear cells were isolated from adult peripheral blood obtained from local routine blood donation by centrifugation over Ficoll Hypaque-1077 gradients. After two washes with HBSS without Ca2+ and Mg2+, monocytes (CD14+ cells) were purified from the PBMCs using an Ab-magnetic bead isolation system (VarioMACS; Miltenyi Biotec). Monocytic THP-1 cells obtained from the American Type Culture Collection (ATCC), were cultured in RPMI 1640 containing 10% FBS.

Measurement of [Ca2+]i

The rhLIGHT-induced rise in [Ca2+]i, was determined using fluo-3/AM and monitored by confocal microscopy (12). In brief, briefly prepared monocytes were incubated in serum-free RPMI 1640 medium with 5 μM of fluo-3/AM at 37°C for 30 min with continuous stirring. After washing with serum-free RPMI 1640 medium, the cells were suspended in PBS containing 0.1% BSA, bound to a poly-L-lysine coated slide for 10 min, and placed in the confocal microscope chamber. PBS containing rhLIGHT was added, and changes in fluorescence every 1.1 s were analyzed with a confocal microscope. The percent killing was calculated as [1-(the number of viable bacteria at time t/the number of viable bacteria at time 0)] × 100.

Immunoprecipitation and immunoblot analysis

THP-1 cells (1 × 107 cells/ml) were incubated with rhLIGHT (1 ng/ml) or HI-rhLIGHT (1 ng/ml) for 10 s and immediately lysed in 500 μl of lysis buffer (20 mM Tris-CI (pH 8.0); 137 mM NaCl; 10% v/v glycerol; 1% v/v Triton X-100; 1 mM Na3VO4; 2 mM EDTA; 1 mM PMSF; 20 μM leupeptin) for 30 min at 4°C. The extracts were preclarred with protein A-Sepharose, followed by immunoprecipitation with 10 μg of anti-PLCγ1 or anti-PLCγ2. Protein A-Sepharose (40 μg) was added to the cell extracts, which were then washed by centrifugation. Protein content was determined by the standard Bradford method. Equal quantities of solubilized proteins were resolved on 10% SDS-PAGE at 20 mA. The separated proteins were transferred to a nitrocellulose membrane and immunoblotted with anti-phosphorytrosine mAb (clone BY2), anti-pTyr771, anti-pTyr788, or anti-pTyr1254 mAb. The blots were then incubated with antigo HRP conjugated to HRP and proteins were detected by ECL (Amersham Biosciences).

Statistics

Differences between groups were evaluated by Student’s t test. Results presented are representatives of at least three independent experiments.

Results

rhLIGHT induces calcium flux in an HVEM-specific manner

First, we investigated whether signaling resulting from the rhLIGHT/HVEM interaction in monocytes was mediated by Ca2+. Monocytes were purified from adult human blood by density gradient centrifugation and isolated with anti-CD14 mAb-Ar Disc originate by centrifugation over Ficoll Hypaque-1077. The percent killing was calculated as [1-(the number of viable bacteria at time t/the number of viable bacteria at time 0)] × 100.

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low levels were present and contributed to the rhLIGHT-induced Ca$^{2+}$ flux. To exclude this possibility, we used HVEM-specific Abs and HSV-1gD to interfere with the binding of rhLIGHT to HVEM. Preincubation of THP-1 cells with anti-HVEM mAbs or HSV-1gD, but not with anti-LTβR mAb, completely blocked the rise in [Ca$^{2+}$]i, indicating that the rhLIGHT-induced Ca$^{2+}$ response was mediated only by HVEM, not LTβR (Fig. 2B). Neither HSV-1gD nor anti-LTβR mAb on their own stimulated [Ca$^{2+}$]i rise in THP-1 cells (Fig. 2B).

To further show that the rhLIGHT-induced Ca$^{2+}$ response was mediated by HVEM, we specifically knocked down HVEM mRNA in THP-1 cells by siRNA, and performed an rhLIGHT-induced calcium response experiment. As shown in Fig. 2C, silencing HVEM significantly attenuated rhLIGHT-induced [Ca$^{2+}$]i rise while calcium responses to fMLP were intact.

The rhLIGHT/HVEM interaction induces tyrosine phosphorylation of PLCγ1

To investigate the mechanism of the rise in [Ca$^{2+}$]i, we tested whether the Ca$^{2+}$ was derived from the medium or intracellular calcium stores. Preincubation of cells with the intracellular calcium chelator, BAPTA-AM, but not with the extracellular calcium chelator, EGTA, blocked rhLIGHT-induced [Ca$^{2+}$]i rise, indicating that the increase in [Ca$^{2+}$]i derived from intracellular stores (Fig. 3A). The rhLIGHT-mediated Ca$^{2+}$ spikes in the presence of extracellular Ca$^{2+}$ were more sustained than those in the absence of extracellular Ca$^{2+}$ (data not shown).

These findings prompted us to investigate the rhLIGHT-induced activation of PLC enzymes using pharmacological inhibitors. As expected, incubation of cells with U73122 or genistein completely

FIGURE 1. rhLIGHT stimulates [Ca$^{2+}$]i rise in monocytes and THP-1 cells. A and B, Freshly isolated human monocytes (A), or log-phase THP-1 cells (B) were stained with fluo-3/AM, attached to glass slides, and placed in a confocal microscope. PBS buffer containing rhLIGHT was added, and changes in fluorescence were monitored every 1.1 s for 100 s. Results are representative of 10 randomly chosen cells. C and D, Mean of the maximum [Ca$^{2+}$]i of ten monocytes (C) and THP-1 cells (D) in response to rhLIGHT. E, THP-1 cells were stimulated with the indicated clonal anti-HVEM mAbs (1 μg/ml), and [Ca$^{2+}$]i, was determined as described for B. F, rhLIGHT induces the bactericidal activities of THP-1 cells. Cells were fed Listeria monocytogenes and treated with rhLIGHT for 10 min. Viable bacteria were determined by plating cell lysates on agar. G, THP-1 cells (2 × 10^6 cells/ml) were cultured with the indicated concentrations of rhLIGHT for 48 h, and TNF-α in culture supernatants was determined by ELISA. Data are means ± SEM. **, p < 0.05; ***, p < 0.01 and ****, p < 0.001 compared with controls. HI, heat inactivated rhLIGHT (1 ng/ml for THP-1 and 10 ng/ml for primary monocytes); BSA, 0.1% BSA-PBS.

FIGURE 2. The rhLIGHT-induced rise of [Ca$^{2+}$]i is mediated by the rhLIGHT/HVEM interaction. A, rhLIGHT (1 ng/ml) was neutralized by incubation with anti-LIGHT mAb (1 μg/ml) at 0°C, for 30 min. The calcium responses of THP-1 cells to control rhLIGHT, neutralized rhLIGHT, HI-rhLIGHT, or BSA were determined. B, THP-1 cells were preincubated with anti-HVEM mAb (3G06), HSV-1gD (gD), or anti-LTβR mAb (α-LT) for 30 min, and rhLIGHT-induced Ca$^{2+}$ responses were measured. Control cells were preincubated with PBS, and gD or α-LT or BSA-induced Ca$^{2+}$ responses were determined. C, THP-1 cells transfected with control siRNA (left panel) or siRNA to HVEM (right panel) were stained with fluo-3/AM, and placed in a confocal microscope. PBS buffer containing 1 ng/ml rhLIGHT was added, and changes in fluorescence were monitored. After 1 min of rhLIGHT addition, PBS containing 1 μg/ml fMLP was added. Results are representative of 10 cells and three independent experiments. Data are means of the maximum [Ca$^{2+}$]i ± SEM of 10 cells. ***, p < 0.001 compared with group of rhLIGHT alone.
abolished rhLIGHT-induced Ca^{2+}/H^{11001} response, while incubation with the U73122 congenor U73343 or pertussis toxin did not, suggesting that the rhLIGHT-induced Ca^{2+}/H^{11001} response was mediated by PLC (Fig. 3B). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate to diacylglycerol and 1,4,5-inositol triphosphate (IP3); the latter binds to the IP3 receptor and regulates the release of Ca^{2+}/H^{11001} from intracellular stores. To confirm that IP3 is produced and is involved in the rhLIGHT-mediated Ca^{2+}/H^{11001} response, we determined whether XeC and 2-APB, antagonists of the IP3 receptor, blocked the response. Fig. 3C shows that this was indeed the case. To further confirm the rhLIGHT-mediated PLC induction, we measured rhLIGHT-induced IP3 production by HPLC: IP3 levels increased dose-dependently in response to rhLIGHT, with peaks 1 min after rhLIGHT addition (Fig. 3D).

Eleven isoforms of PLC, grouped into four subfamilies (β, γ, δ, and ε), have been identified in mammals. Two isoforms of PLCγ (γ1 and γ2) are phosphorylated and activated after ligands bind to nonreceptor protein tyrosine kinases, such as membrane IgM in B

**FIGURE 3.** rhLIGHT increases [Ca^{2+}/H^{11001}] in THP-1 cells via PLC activation. A, rhLIGHT-induced [Ca^{2+}/H^{11001}] is derived from intracellular Ca^{2+} stores. THP-1 cells were preincubated with PBS containing BAPTA-AM (1, 2.5, 10 μM) or EGTA (2 μM) for 30 min, stained with fluo-3, attached to a glass slide, placed in a confocal microscope and the rhLIGHT-induced Ca^{2+}/H^{11001} response was determined. B, PLC inhibitors inhibit rhLIGHT-induced Ca^{2+}/H^{11001} response. THP-1 cells were preincubated with U73122 (0.1, 1, and 3 μM), U73343 (3 μM), or genistein (100 μM) for 30 min, or with pertussis toxin (PTX, 100 ng/ml) for 120 min, and rhLIGHT-induced rise of [Ca^{2+}/H^{11001}] was determined by confocal microscopy. C, IP3 receptor antagonists inhibit rhLIGHT-induced Ca^{2+}/H^{11001} response. THP-1 cells were preincubated with XeC (2 and 4 μM) or 2-APB (10 and 100 μM) for 30 min, and rhLIGHT-induced rise of [Ca^{2+}/H^{11001}] was determined as described in A. The mean of the maximum [Ca^{2+}/H^{11001}], of ten cells is shown. D, Dose- and time-dependent rhLIGHT-mediated IP3 productions. THP-1 cells were stimulated with the indicated concentrations of rhLIGHT for 1 min (left panel) or with 1 ng/ml rhLIGHT for the indicated times (right panel). Intracellular IP3 was measured as described in Materials and Methods.

**FIGURE 4.** rhLIGHT induces phosphorylation of PLCγ1 in THP-1 cells. A, Cells were preincubated with anti-HVEM mAb (3G06) or control mouse IgG1 for 30 min, and stimulated with rhLIGHT (1 ng/ml) or HI-rhLIGHT (1 ng/ml) for 10 s. Cell lysates were prepared, immunoprecipitated with anti-PLCγ1 or anti-PLCγ2 mAb, resolved on SDS-PAGE, and immunoblotted with antiphosphotyrosine mAb. B, left, Samples from A were immunoblotted with site-specific anti-pTyr771, anti-pTyr783 and anti-pTyr1254 mAb. Right, COS-7 cells were stimulated with 0, 1, 10, or 100 ng of recombinant human epidermal growth factor (rhEGF) for 1 min, immunoprecipitated with anti-PLCγ1 mAb, and blotted with anti-Tyr771, Tyr783, or Tyr1254 mAb. C, THP-1 cells transfected with PLCγ1 siRNA are impaired in rhLIGHT-induced calcium responses. THP-1 cells transfected with control siRNA (top panel) or siRNA to PLCγ1 (middle and bottom panel) were attached to glass slides, incubated with fluo-3/AM, and placed in a confocal microscope. PBS buffer containing 1 ng/ml rhLIGHT was added, and changes in fluorescence were monitored. After 1 min of rhLIGHT addition, PBS containing 1 μM of A23187 or 1 μg/ml fMLP was added. Results are representative of 10 cells and three independent experiments.
rhLIGHT treatment led to degradation of IκBα. A, THP-1 cells were stimulated with rhLIGHT (0, 1, 10, or 100 ng/ml) for 30 min. Cell lysates were prepared, resolved on SDS-PAGE, and immunoblotted with anti-IκBα mAb. IκBα levels were quantified and normalized to actin in three independent experiments and the graph shows mean ± SDs. B, THP-1 cells were pretreated with U73122, BAPTA-AM, or XeC (4 μM) for 30 min and stimulated with 1 ng/ml rhLIGHT for 30 min. Cell lysates were prepared, and immunoblotted with anti-IκBα mAb as in A.

cells, the TCR in T cells, FcεR in basophils, and FcγR in monocytes (17). To see whether the rhLIGHT/HVEM interaction also activated and phosphorylated PLCγ in monocytes, THP-1 cells were treated with rhLIGHT, and lysates were immunoprecipitated with anti-PLCγ1 or anti-PLCγ2 mAb and blotted with anti-phosphotyrosine mAb. rhLIGHT induced tyrosine phosphorylation of PLCγ1, but not PLCγ2 (Fig. 4A). PLCγ1 can be phosphorylated on residues Tyr771, Tyr783, and Tyr1254 (18). Lysates of rhLIGHT-treated THP-1 cells were immunoprecipitated with anti-PLCγ1 and blotted with site-specific anti-phosphotyrosine mAbs. Only Tyr771 of PLCγ1 was phosphorylated by HVEM activation (Fig. 4B).

To further establish PLCγ1 as a downstream signaling molecule of the rhLIGHT/HVEM interaction, we knocked down PLCγ1 mRNA in THP-1 cells with siRNA. As shown in Fig. 4C, silencing PLCγ1 completely abolished rhLIGHT-induced [Ca²⁺], rise while responses to A23187 or fMLP remained intact.

The rhLIGHT/HVEM interaction induces IκBα degradation, which is blocked by pharmacological PLC/Ca²⁺ inhibitors

rhLIGHT-induced NFκB activation and cytokine production have been demonstrated in T cells (4). We tested for rhLIGHT-induced NFκB activation and examined its relation to PLC/Ca²⁺ signaling by measuring IκBα in THP-1 cells. Cells were stimulated with rhLIGHT and lysates were blotted with anti-IκBα mAb. Fig. 5A shows that rhLIGHT treatment led to degradation of IκBα. Moreover, preincubation of THP-1 cells with U73122, XeC, or BAPTA-AM completely blocked this rhLIGHT-induced IκBα degradation (Fig. 5B), indicating that the PLC/Ca²⁺ signaling lies up-stream of NFκB activation.

Inhibitors of PLC/Ca²⁺ signaling block rhLIGHT-mediated TNF-α and ROS production, and the bactericidal activity of monocytes

Monocytes are the primary lines of defense against bacterial pathogens. They phagocytose and kill bacteria by producing ROS and inflammatory cytokines such as TNF-α. We have shown that the rhLIGHT/HVEM interaction causes the production of inflammatory cytokines and ROS, and enhances bactericidal activities in human primary monocytes (8). In the present study, we showed that inhibitors of PLC/Ca²⁺ blocked these responses; preincubation of purified monocytes with genistein, U73122, XeC, or BAPTA-AM dose-dependently suppressed the production of TNF-α (Fig. 6A) and ROS (Fig. 6B), and the increase of bacterial killing activity (Fig. 6C).

Inhibitors of the PLC/[Ca²⁺], signaling cascade abolish rhLIGHT-induced monocyte activation. A, Freshly isolated human monocytes were preincubated with genistein, U73122, U73343 (3 μM), XeC, or BAPTA-AM for 30 min, washed, and cultured with 10 ng/ml rhLIGHT for 24 h. TNF-α in the culture supernatants was determined by ELISA. B, Monocytes were preincubated with inhibitors as in A, allowed to phagocytose Listeria monocytogenes and treated with 10 ng/ml rhLIGHT for 10 min. The number of viable bacteria remaining in the cells was determined by serial plating of cell lysates on agar plates. Data are means ± SEM, and are representative of three similar experiments. *, p < 0.05; **, p < 0.01; and ***, p < 0.001, compared with group of rhLIGHT alone.

Discussion

Recently we reported that HVEM stimulation by rhLIGHT enhanced the bactericidal activities of human monocytes and neutrophils against L. monocytogenes and S. aureus (8). In this study, we investigated the signaling pathways evoked by the LIGHT/HVEM interaction. rhLIGHT as well as anti-HVEM mAb produced a rapid [Ca²⁺], rise in both primary monocytes and THP-1 cells (Fig. 1). Treatment of THP-1 cells with pharmacological inhibitors of the PLC/Ca²⁺ pathway and with siRNA to PLCγ1 completely abolished the [Ca²⁺], elevation (Fig. 3), and the rhLIGHT stimulus resulted in IP₃ production and specific phosphorylation of PLCγ1 on Tyr771. Further observations showed that the LIGHT/HVEM interaction acts via a PLC/Ca²⁺ signaling cascade.

LIGHT binds to three molecules, HVEM, LTβR, and Drβ3 (2). However, there is good evidence that the events considered in this study result from specific binding of rhLIGHT to HVEM. First, HVEM is the only receptor for LIGHT in monocytes; LTβR is not expressed in monocytes (8) and Drβ3 is a soluble protein not present on membranes. Second, preincubation of cells with HSV-1gD, which specifically binds to HVEM, not to LTβR or Drβ3,
abolished rhLIGHT-induced Ca\textsuperscript{2+} response (Fig. 2B). Third, rhLIGHT-induced Ca\textsuperscript{2+} response was blocked by preincubation of cells with antagonistic anti-HVEM mAb, but not anti-LT\textbeta R mAb (Fig. 2B). Moreover, agonistic Abs specific for HVEM also induced Ca\textsuperscript{2+} influx (Fig. 1E) and silencing HVEM mRNA abolished the rhLIGHT-induced calcium responses (Fig. 2C).

Both PLC\textgamma 1 and PLC\textgamma 2 are present in immune cells and play critical roles in the generation of calcium signals in response to stimulation of immune receptors. The signaling mechanisms of PLC\textgamma 1 and PLC\textgamma 2 are similar, in that both isoforms are phosphorylated and are targets of the same protein tyrosine kinases (19). However, most immune cells use only one isoform for specific immune receptor activation. For example, only PLC\textgamma 1 is activated by TCR activation in T cells, while PLC\textgamma 2 is activated by the BCR complex in B cells and by collagen receptor glycoprotein VI in platelets (19). We showed in this study that HVEM activation only leads to phosphorylation of PLC\textgamma 1 not of PLC\textgamma 2 (Fig. 4A).

It has been shown that phosphorylation of only a small fraction of the expressed PLC\textgamma is enough to induce normal Ca\textsuperscript{2+} responses (20). Therefore, the preference of PLC\textgamma 1 in HVEM signaling in monocytes may not be due to the greater expression of PLC\textgamma 1 but to differential affinities of the HVEM signaling molecules for PLC\textgamma 1 or for the protein tyrosine kinases that phosphorylate PLC\textgamma 1. It is known that Src, Syk, and Tec protein tyrosine kinases are present in monocytes and phosphorylate PLC\textgamma upon activation of specific immune receptors (21–23).

PLC\textgamma 1 contains many protein binding domains: two Src-homology domain (SH)2, one SH3, and two pleckstrin homology domains, as well as two catalytic domains, and binds to many proteins via these domains. For example, the pleckstrin homology domains of PLC\textgamma 1 bind the nonreceptor tyrosine kinase protein tyrosine phosphatase 1C (PTP1C) (24), which regulates PLC\textgamma 1 activity (25). In the case of PLC\textgamma 1, these tyrosine kinases are known to inhibit PLC\textgamma 1 activity (26). The precise mechanism of PLC\textgamma 1 activation is unknown, the results of this study indicate that rhLIGHT/HVEM signaling in monocytes mobilizes Ca\textsuperscript{2+} via PLC\textgamma 1.

Acknowledgments

We thank members of the Ulsan Red Cross Blood Center for supplying human peripheral blood samples.

Disclosures

The authors have no financial conflict of interest.

References

not IRGC. Furthermore, although the statement on page 7197 “Humans have only one intact p47 GTPase (IRGC) whose expression has been reported from testis but not THP-1 cells” is correct, it has little relevance to our work because we only measured the transcript of IRGM.


The authors apologize for any confusion caused and are grateful to colleagues in the field for bringing the errors to their attention. In particular, the authors wish to acknowledge Dr. Jonathan Howard of the University of Cologne Institute for Genetics (Cologne, Germany) for pointing out these errors and for helpful discussion and advice on preparing the erratum. Overall, the findings with IRGM were modest and do not form a major part of the conclusions, particularly those concerning Cathelicidin LL-37 and its role in resistance to tuberculosis.


In Footnotes, the country listed for grant support is incorrect. The footnote should read: 1 This work was supported by grant 2005-SGR00037 from the Generalitat de Catalunya, Spain.

In Results, under the heading Participation of gray cells in cytotoxic contact reactions, “archaeocytes” is misspelled in the fourth sentence of the first paragraph. The sentence should read: “However, it is likely that this migration of archaeocytes has as a final outcome their differentiation into gray cells (Fig. 2C) rather than a direct action of their own (7).”


In Materials and Methods, under the heading Statistical analysis on page 5979, the phrases “within each individual” and “within individuals” in the last two sentences of the first paragraph are incorrect. The sentences should read: “A two-tailed paired Student’s t test was used to compare response between NK cells expressing different numbers of S-KIR. The paired Student’s t test was also used to compare percentages of KIR-expressing NK subgroups.”


There is an error in the affiliation line for the seventh author due to an incorrect symbol. The correct affiliations for Byoung S. Kwon are: 2Department of Biomedicine and 3Immunomodulation Research Center, University of Ulsan, Ulsan, South Korea.