CD38 is an ADP-ribosyl cyclase, producing a potent Ca\textsuperscript{2+} mobilizer cyclic ADP-ribose (cADPR). In this study, we have investigated a role of CD38 and its regulation through interleukin-8 (IL8) signaling in lymphokine-activated killer (LAK) cells. Incubation of LAK cells with IL8 resulted in an increase of cellular cADPR level and a rapid rise of intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}])\textsubscript{i}, which was sustained for a long period of time (>10 min). Preincubation of an antagonistic cADPR analog, 8-Br-cADPR (8-bromo-cyclic adenosine diphosphate ribose), abolished the sustained Ca\textsuperscript{2+} signal only but not the initial Ca\textsuperscript{2+} rise. An inositol 1,4,5-trisphosphate (IP\textsubscript{3}) receptor antagonist blocked both Ca\textsuperscript{2+} signals. Interestingly, the sustained Ca\textsuperscript{2+} rise was not observed in the absence of extracellular Ca\textsuperscript{2+}. Functional CD38-null (CD38\textsuperscript{-/-}) LAK cells showed the initial rapid increase of [Ca\textsuperscript{2+}])\textsubscript{i} but not the sustained Ca\textsuperscript{2+} rise in response to IL8 treatment. An increase of cellular cADPR level by cGMP analog, 8-pCPT-cGMP (8-(4-chlorophenylthio)-guanosine-3',5'-cyclic monophosphate), but not cAMP analog or phorbol 12-myristate 13-acetate was observed. IL8 treatment resulted in the increase of cGMP level that was inhibited by the IP\textsubscript{3} receptor blocker but not a protein kinase C inhibitor, cGMP-mediated Ca\textsuperscript{2+} rise was blocked by 8-Br-cADPR. In addition, IL8-mediated LAK cell migration was inhibited by 8-Br-cADPR and a protein kinase G inhibitor. Consistent with these observations, IL8-induced migration of CD38\textsuperscript{-/-} LAK cells was not observed. However, direct application of cADPR or 8-pCPT-cGMP stimulated migration of CD38\textsuperscript{+} cells. These results demonstrate that CD38 is stimulated by sequential activation of IL8 receptor, IP\textsubscript{3}-mediated Ca\textsuperscript{2+} rise, and cGMP/protein kinase G and that CD38 plays an essential role in IL8-induced migration of LAK cells.

A type II transmembrane protein CD38, originally known as an activation antigen, displays ADP-ribosyl cyclase (ADPR-cyclase)\textsuperscript{1} and cyclic ADP-ribose hydrolase (cADPR-hydrolase) activities (1, 2). These two enzyme activities are involved in the conversion of β-nicotinamide adenine dinucleotide (β-NAD\textsuperscript{+}) first to cyclic ADP-ribose (cADPR) and then to ADP-ribose (ADPR) (3–5). CD38 is also ADP-ribosylated by ecto-ADP-ribosyltransferase in the presence of exogenous β-NAD\textsuperscript{+} (6). This modification results in inactivation of the enzyme activity. The metabolite cADPR is known to induce Ca\textsuperscript{2+} release from intracellular stores by acting on ryanodine receptor and/or Ca\textsuperscript{2+} influx through plasma membrane Ca\textsuperscript{2+} channels in a variety of cells (7–11). Several studies have indicated that cADPR synthesis by CD38 is stimulated through cell surface heterotrimeric G-protein-coupled receptor signaling. The receptors include β-adrenergic receptor in cardiac myocytes (12) and artery smooth muscle cells (13), angiotensin II receptor in cardiac myocytes (14), muscarinic receptor in neuroblastoma NG-108 (15), and pancreatic acinar cells (16). The activation of ADPRcyclase by cGMP in Aplysia californica has been reported (17), and cAMP-dependent activation of the enzyme is also observed in artery smooth muscle cells (13). However, the molecular basis of the activation of CD38 and/or ADPR-cyclases has not been clearly defined.

A previous report has indicated that a peptide cytokine interleukin-8 (IL8) signaling may utilize cADPR to mobilize Ca\textsuperscript{2+}, in IL2-activated natural killer cells (18). IL8, which belongs to the CXC superfamily of chemokines, plays an important role in the motility of various cells such as neutrophils and T cells (19, 20) and also induces angiogenesis and other effects associated with proinflammatory responses (21–23). The IL8 receptor (IL8R) is made of seven transmembrane proteins and couples with G\textsubscript{i} and stimulates the production of 1,4,5-trisphosphate (IP\textsubscript{3}) through the activation of phospholipase C (PLC)-β2 (24, 25). There is a report that the IL8Rs present in natural killer cells and lymphokine-activated killer (LAK) cells may induce cADPR synthesis through the G\textsubscript{i}-involved signaling pathway (26). CD38 expression in natural killer and LAK cells is also observed (27, 28). However, the role of CD38 including cADPR in LAK cell functions and the activation pathways of CD38 remain elusive.

In this study, we have investigated IL8-mediated regulation of CD38 by determining intracellular Ca\textsuperscript{2+} changes and motility of LAK cells. The results indicate that CD38 is activated via
cGMP/protein kinase G (PKG) that is activated by IL8 and that CD38 plays a critical role in IL8-mediated Ca^{2+} signal and migration of LAK cells.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Antibodies were obtained as follows: anti-human CD38 monoclonal antibody from BD Biosciences; anti-IL-8 monoclonal antibody from Santa Cruz Biotechnology (Santa Cruz, CA); anti-PLC-β2 monoclonal antibody from P. G. Suh at POSTECH (Pohang, Korea); anti-IP3 receptor (IP3R) generously provided by S. H. Kim (Inha University, Incheon, Korea); anti-ryanodine receptor (RyR) from Amsbergs Biosciences. Horseradish peroxidase-conjugated anti-rabbit IgG was purchased from Advanced Biologicals Inc. (Jeonju, Korea). Human recombinant IL2 was obtained from Chiron BV (Amsterdam, Netherlands). Human recombinant IL6, human AB serum, and all other reagents were obtained from Sigma. RPMI 1640 was from Invitrogen.

**Preparation of LAK Cells**—LAK cells were prepared as described previously (29, 30). Briefly, blood obtained from healthy volunteers was layered over Ficoll-Hypaque and centrifuged at 700 × g for 30 min to remove red blood cells. After centrifuged red blood cells preparation were incubated on a nylon-coated nylon for 3 h at 37 °C in a 5% CO2 incubator to remove B lymphocytes and macrophages. Nylon-coated non-adherent cells were collected and further separated by a Percoll density gradient centrifugation. Four layers of Percoll were used: 37, 44, 52, and 60%. After centrifugation at 700 × g for 20 min, cells of the 52% Percoll layer were collected, washed with serum-free RPMI 1640, and incubated at 37 °C for 2 h to remove all contaminating nucleotides. The samples were treated with 0.5 M perchloric acid under sonication. Precipitates were removed by centrifugation at 20,000 × g for 10 min. Perchloric acid was removed by mixing the aqueous sample with a solution containing 3 volumes of 1,1,2-trichlorotrifluoroethane to 1 volume of tri-n-octylamine. After centrifugation for 10 min at 1500 × g, the aqueous layer was collected and neutralized with 20 mM sodium phosphate (pH 8). To remove all contaminating nucleotides, the samples were incubated with the following hydrolytic enzymes overnight at 37 °C: 0.44 unit/ml nucleotide pyrophosphatase, 12.5 units/ml alkaline phosphatase, 0.0625 units/ml NAD+ and 0.025 unit/ml ADP-ribosidase in 20 mM sodium phosphate buffer (pH 8.0). Enzymes were removed by filtration using Centricon-3 filters. To convert cADPR to NAD{\textsuperscript+}, the samples (0.1 ml/tube) were incubated with 50 μl of a cycling reagent containing 0.3 μM/ml Aplysia ADPR-cyclase, 30 mM nicotinamide, and 100 mM sodium phosphate (pH 8) at room temperature for 30 min. The samples were further incubated with the cycling reagent (0.1 ml) containing 2% ethanol, 100 μg/ml alcohol dehydrogenase, 20 μg/ml resazarin, 10 μg/ml diaphorase, 5 mM phosphate, 10 μg/ml bovine serum albumin, 0.1 M riboflavin, and 100 mM sodium phosphate (pH 8.0) for 2 h at room temperature. An increase in the resorufin fluorescence was measured at 544 nm excitation and 590 nm emission using a fluorescence plate reader (Molecular Devices Corp., Spectra-Max GEMINI). Various concentrations of cADPR were also included in the cycling reaction to generate a standard curve.

**Measurement of Intracellular cGMP Level**—Levels of cGMP were determined by a [\textsuperscript{3}H]-cGMP radioimmunoassay kit according to the manufacturer’s protocol. LAK cells were preincubated with 0.5 μM ibutylmethylxanthine and phosphodiesterase inhibitor and then challenged with various reagents as detailed in the figure legends. After incubation for 30 min, cells were treated with equal volume of 12% trichloroacetic acid. To determine cGMP extraction efficacy, [%HcGMP (1500 pm)] was added. After centrifugation at 20,000 × g for 3 min, supernatants were collected and extracted using a clayer portion of water-saturated diethyl ether. The water layer was collected, dried using SpeedVac, and dissolved in 200 μl of 50 mM sodium acetate buffer (pH 6.2). Prior to performing radioimmunoassay, the sample (100 μl) was acetylated using acetic anhydride in the presence of triethylamine. A standard curve of acetylated cGMP was also prepared as described in the manufacturer’s protocol.

**Measurement of [Ca^{2+}]_{i}**—LAK cells were washed with Hank’s balanced salt solution (2 mM CaCl{\textsubscript{2}}, 145 mM NaCl, 5 mM KCl, 1 mM MgCl{\textsubscript{2}}, 5 mM glucose, 20 mM HEPES, pH 7.3) containing 1% BSA and incubated in the same solution containing 1% BSA at 37 °C for 40 min. The cells were washed three times with Hank’s balanced salt solution. Changes in [Ca^{2+}]_{i} in LAK cells were determined at 488 nm excitation/530 nm emission by air-cooled argon laser system (34). The emitted fluorescence at 530 nm was collected using a photomultiplier. One image every 6 s for 10 min was scanned using confocal microscope (Nikon, Japan). For the calculation of [Ca^{2+}]_{i}, the method of Tsien et al. (35) was used with the following equation: [Ca^{2+}]_{i} = [K_{F} \cdot F_{\text{max}} - F_{\text{background}}] / K_{f} \cdot F_{\text{max}} - F_{\text{background}}

**Statistical Analysis**—Data represent means ± S.E. of the mean (S.E.) of at least three separate experiments. Statistical analysis was performed using Student’s t-test. A value of p < 0.05 was considered significant.
Activation of CD38 by IL8 Signaling in LAK Cells

**RESULTS**

Induction of CD38 Expression in LAK Cells—Firstly, we assessed expression levels of CD38 along with the other related signaling molecules during induction of LAK cells by IL2 treatment. Expression of CD38 was gradually increased in a time-dependent manner and highly induced at 7–10 days (Fig. 1A). Expression of RyR, a putative receptor for cADPR, was also highly induced at 7–10 days. On the other hand, expression of IL8R, IP3R, and PLC-γ2 was observed in the freshly isolated T cells and was not influenced by IL2 treatment (data not shown). Expression of CD38 was ascertained by measuring ADPR-cyclase activity using NGD⁺, which is converted to cGMP only by the enzyme (Fig. 1B). Consistent with the above observations, the production of cADPR in cell lysates was increased in a time-dependent manner. Next, we examined whether IL8 stimulates CD38 in LAK cells induced by treatment with IL2 for 10 days. As shown in Fig. 1C, [cADPR], in LAK cells was increased significantly by the treatment with IL8. These results show that expression of CD38 and RyR along with transformation of T cells to LAK cells are induced by IL2 treatment and that IL8 signaling may activate CD38.

IL8-mediated Elevation of [Ca²⁺]i Involves Activation of CD38—On the basis of the above observation that IL8 treatment increases [cADPR], the molecular basis of CD38 activation by IL8 in LAK cells was examined by determining IL8-mediated changes in [Ca²⁺], under various conditions. The addition of IL8 to the cells resulted in a rapid increase in [Ca²⁺], and the increased [Ca²⁺], levels were sustained for more than 10 min (Fig. 2A). The pretreatment of an antagonistic cADPR analog, 8-Br-cADPR, abolished the IL8-mediated sustained Ca²⁺ signal but not the initial rapid increase (Fig. 2B). Interestingly, xestospongin C, an IP3 receptor antagonist, completely abolished both Ca²⁺ signals (Fig. 2C). In contrast, calphostin C, a protein kinase C inhibitor, had no effect on the IL8-mediated increase of [Ca²⁺]i (Fig. 2D). To examine whether the sustained Ca²⁺ increase is due to Ca²⁺ release from the intracellular store or Ca²⁺ influx, the IL8-mediated increase of [Ca²⁺]i was determined in the presence of EGTA. The sustained Ca²⁺ rise was not observed while the initial Ca²⁺ increase was present (Fig. 2E). The effects of various agents on the initial and sustained Ca²⁺ increases were summarized in Fig. 2, F and G, respectively. These results indicate that cADPR is responsible for IL8-mediated sustained Ca²⁺ influx that is dependent on the IP3-mediated initial Ca²⁺ rise in the LAK cells.

Inactivation of CD38 by ADP-ribosylation Abolishes the Sustained Rise of [Ca²⁺], Induced by IL8—We have previously demonstrated that CD38 in the activated T cells is ADP-ribosylated in the presence of exogenous β-NAD⁺, resulting in a loss of enzyme activity (6). To further elucidate the role of CD38/cADPR in IL8-induced Ca²⁺ signaling, functional CD38-null (CD38−/−) LAK cells were prepared by treatment with β-NAD⁺. As shown in Fig. 3A, ADPR-cyclase activities, which were observed in the lysates and CD38 immunoprecipitates prepared from the control LAK cells, were completely eradicated by the incubation with β-NAD⁺. These results indicated that CD38 represents the only ADPR-cyclase in LAK cells but also indicate that CD38 is inactivated in the presence of β-NAD⁺. When IL8-mediated Ca²⁺ signal in CD38−/− LAK cells was compared with the control LAK cells (Fig. 3B), CD38−/− LAK cells showed only the initial rise of [Ca²⁺], but not the sustained rise of [Ca²⁺]i by the treatment with IL8 (Fig. 3C). To ensure that the sustained Ca²⁺ signal is mediated by cADPR, changes of [Ca²⁺]i in the CD38−/− LAK cells were determined in the presence of cADPR. Indeed, cADPR was able to induce the sustained Ca²⁺ signal (Fig. 3D). As summarized in Fig. 3, E and F, the sustained Ca²⁺ increase is due to the activation of CD38/cADPR through IL8 signaling.

CD38 Is Activated by cGMP Produced by IL8/Ca²⁺ Signaling—It has been demonstrated that ADPR-cyclases including CD38 are probably activated by cAMP or cGMP (13, 17). Stimulation of IL8R produces two second messengers, IP3 and diacylglycerol. There is a report that IL8R may couple with Gs protein and that IL8 signaling may activate CD38. Consistent with previous observations (6), when IL8-mediated Ca²⁺ signal in CD38−/− LAK cells was compared with the control LAK cells (Fig. 3B), CD38−/− LAK cells showed only the initial rise of [Ca²⁺], but not the sustained rise of [Ca²⁺]i by the treatment with IL8 (Fig. 3C). To ensure that the sustained Ca²⁺ signal is mediated by cADPR, changes of [Ca²⁺]i in the CD38−/− LAK cells were determined in the presence of cADPR. Indeed, cADPR was able to induce the sustained Ca²⁺ signal (Fig. 3D). As summarized in Fig. 3, E and F, the sustained Ca²⁺ increase is due to the activation of CD38/cADPR through IL8 signaling.

**FIG. 1.** Expression of CD38 and RyR during induction of LAK cells. Isolated T lymphocytes were treated with IL2 for 10 days. During induction of LAK cells, expression of CD38 and RyR was determined. A, CD38 and RyR are expressed during induction of LAK cell by IL2. A typical Western blot of CD38 and RyR expression during induction of LAK cells is shown. Cell lysate (20 μg) was subjected to immunoblotting using CD38 and RyR antibodies. B, increase of ADPR activity during LAK cell induction. ADPR-cyclase activity in cell lysates (10 μg) prepared from IL2 treated cells was determined using 200 μm NGD⁺ as a substrate. NA, no activity found. C, [cADPR], is increased in response to IL8 treatment. LAK cells were incubated with IL8 (10 ng) for 1.5 min. Formation of cADPR was determined as described under “Experimental Procedures.” The data ± S.E. from three independent experiments are shown. **, p < 0.005.
not increased by the ionomycin treatment. The observations that initial Ca\(^{2+}\) rise proceeded to increase the sustained Ca\(^{2+}\) signal (Fig. 2, A and B) indicated that cGMP increase induced by IL8 may proceed to form cADPR. The time course of cGMP and cADPR formation induced by IL8 was evaluated. The results showed that levels of cGMP and cADPR were increased in a time-dependent manner, reaching maximal levels at 60 and 90 s, respectively (Fig. 4 C). The formation of cGMP was rapidly reduced, whereas the formation of cADPR was slowly decreased. Supporting these results, a cGMP analog, 8-pCPT-cGMP, generated the sustained increase of [Ca\(^{2+}\)], (Fig. 4D). The cGMP analog-mediated increase of [Ca\(^{2+}\)], was completely blocked by pretreatment with 8-Br-cADPR (Fig. 4E) but not by xestospongin C (Fig. 4F). Moreover, Rp-8-pCPT-cGMPS blocked IL8-induced sustained Ca\(^{2+}\) rise but not the initial Ca\(^{2+}\) rise (Fig. 4G). Differences in [Ca\(^{2+}\)], during initial and sustained increases of [Ca\(^{2+}\)], were summarized in Fig. 4, H and I, respectively. These findings indicate that cGMP is involved in the activation of CD38 in LAK cells and that IL8-induced cGMP formation is due to IP3-mediated Ca\(^{2+}\) rise. In addition, we also assessed whether phosphorylation of CD38 via cGMP/PKG during IL8 treatment occurred. Phosphorylation of CD38 was not observed. The increased production of cGDPR by CD38 isolated from LAK cells by immunoprecipitation was not observed in the presence of cGMP, PKG, or both together or Ca\(^{2+}\) (data not shown).

**cADPR is involved in IL8-mediated increase in [Ca\(^{2+}\)].** A, the treatment of LAK cells with IL8 (10 pm) induces long-lasting intracellular Ca\(^{2+}\) signal. B, antagonistic cADPR analog abolishes the IL8-mediated sustained Ca\(^{2+}\) increase, whereas the initial Ca\(^{2+}\) increase remains. LAK cells were preincubated with 8-Br-cADPR (100 pm) for 30 min, and IL8 (10 pm) was then added to the cells. C, IP\(_3\)R blocker completely eliminates IL8-induced Ca\(^{2+}\) signals. LAK cells were preincubated with xestospongin C (2 pm) for 30 min. D, protein kinase C inhibitor does not block IL8-mediated increase of [Ca\(^{2+}\)]. Prior to treatment with IL8, xestospongin C (100 pm) was preincubated at 37 °C for 30 min. E, IL8-mediated sustained Ca\(^{2+}\) rise is due to Ca\(^{2+}\) influx. IL8-mediated Ca\(^{2+}\) signals were determined in the presence of 3 mm EGTA (Ca\(^{2+}\)-free). Three representative Ca\(^{2+}\) traces are shown. Arrows indicate the time point of the addition of IL8. F, a direct comparison of mean [Ca\(^{2+}\)], during initial increases of [Ca\(^{2+}\)]. The data shown are analyzed at 54 s. #, buffer versus IL8 or IL8 plus agents indicated, p < 0.001; ***, IL8 versus xestospongin C plus IL8, p < 0.001. G, a direct comparison of mean [Ca\(^{2+}\)], during sustained increases of [Ca\(^{2+}\)]. The data shown are analyzed at 300 s. #, buffer versus IL8 or calphostin C plus IL8, p < 0.001; ** and ***, IL8 versus IL8 plus agents indicated, p < 0.001 and p < 0.005, respectively. Cell numbers are presented in the parentheses. Data are mean ± S.E.

**cGMP/PKG/cADPR Signaling System Is Essential for IL8-induced Migratory Activity of LAK Cells**—It is well known that the activation of IL8R induces migration of various cell types (19, 20). To examine whether CD38/cADPR plays any role in IL8-mediated cell migration, we evaluated the effect of cADPR on the migratory activity of LAK cells. As presented in Fig. 5A, LAK cell migration was significantly induced by IL8. The IL8-mediated cell migration was blocked by pretreatment with
8-Br-cADPR. In addition, we also examined the effects of Ca\(^{2+}\)/H\(_{11001}\) on LAK cell migration (Fig. 5A). IL8-induced cell migration was not observed in the absence of extracellular Ca\(^{2+}\), and the increase of [Ca\(^{2+}\)/H\(_{11001}\)]\(_i\) by ionomycin did not induce cell migration.

The above observations that CD38/cADPR is involved in LAK cell migration were further examined using CD38\(^{-}\)LAK cells. IL8-mediated migration of CD38\(^{-}\)LAK cells was not observed (Fig. 5B). However, the treatment of CD38\(^{-}\) LAK cells with exogenous cADPR stimulated cell migration similar to the control LAK cells induced by IL8. To further support the observation that cGMP is able to activate CD38, cGMP-mediated LAK cell migration was examined. An agonistic membrane-permeable cGMP analog, 8-pCPT-cGMP, stimulated the migratory activity of LAK cells independent of the activation of IL8R (Fig. 5C).

The pretreatment of PKG inhibitor, Rp-8-pCPT-cGMPS, completely blocked IL8-induced migration of LAK cells. These results indicate that CD38/cADPR plays an important role in IL8-mediated migration of LAK cells.

**DISCUSSION**

CD38 is a bifunctional enzyme having ADPR-cyclase and hydrolase activity that produces and hydrolyzes cADPR, which is a powerful and universal Ca\(^{2+}\)-mobilizing second messenger. Studies have proposed that CD38, including ADPR-cyclases, is activated by G-protein-coupled receptor. However, the regulation pathway(s) of CD38 by G-protein-coupled receptor remains unclear. In this study, we for the first time demonstrate that CD38 induced in LAK cells is stimulated by cGMP/PKG that is generated upon the activation of IL8R. Our results have also revealed that a CD38 metabolite, cADPR, plays an essential role in regulation of [Ca\(^{2+}\)/H\(_{11001}\)]\(_i\) and migratory activity of LAK cells.
producing two second messengers, IP3 and diacylglycerol (24, 25). The IL8 treatment of LAK cells exhibits a rapid rise of \([\text{Ca}^{2+}]_i\) that sustains for a long period of time (>10 min). Our results show that the initial \([\text{Ca}^{2+}]_i\) signal is mediated by IP3 and that the IL8-mediated sustained \([\text{Ca}^{2+}]_i\) signal is due to the activation of CD38, resulting in an increase of [cADPR]. Thus, the pretreatment of cells with 8-Br-cADPR, an antagonistic analog of cADPR, displays only the initial rise of \([\text{Ca}^{2+}]_i\). A n IP3R blocker, xestospongin C, abolishes completely the IL8-mediated elevation of the initial and sustained \([\text{Ca}^{2+}]_i\), indicating that the activation of CD38 requires the IP3-mediated increase of \([\text{Ca}^{2+}]_i\). However, in contrast to IL8-induced forma-
Activation of CD38 by IL8 Signaling in LAK Cells

A. CD38/cADPR mediates IL8-stimulated migratory activity of LAK cells. A, IL8-induced stimulation of LAK migration is blocked by antagonistic cADPR analog 8-Br-cADPR. Cells treated with 100 μM 8-Br-cADPR for 30 min were incubated with IL8 (10 pM). Hanks’ balanced salt solution containing 3 mM EGTA was used to deplete extracellular Ca\(^{2+}\), and ionomycin was 200 nM. The cell migration assay was performed as detailed under “Experimental Procedures.” *, \(p < 0.05\); **, \(p < 0.005\). B, IL8-induced migratory activity of CD38− LAK cells is absent. Incubation of CD38− LAK cells with cADPR (250 μM) stimulates cell migration. *, \(p < 0.05\); **, \(p < 0.005\). C, cGMP/PKG stimulates migration of LAK cells. Cells pretreated with Rp-8-pCPT-cGMPS (20 μM) for 30 min were incubated with IL8 (10 pM). For the determination of cGMPS-mediated cell migration, the cell-permeable cGMP analog, 8-pCPT-cGMP (1 mM), was used. **, \(p < 0.005\).

Fig. 5. CD38/cADPR mediates IL8-stimulated migratory activity of LAK cells. A, IL8-induced stimulation of LAK migration is blocked by antagonistic cADPR analog 8-Br-cADPR. Cells treated with 100 μM 8-Br-cADPR for 30 min were incubated with IL8 (10 pM). Hanks’ balanced salt solution containing 3 mM EGTA was used to deplete extracellular Ca\(^{2+}\), and ionomycin was 200 nM. The cell migration assay was performed as detailed under “Experimental Procedures.” *, \(p < 0.05\); **, \(p < 0.005\). B, IL8-induced migratory activity of CD38− LAK cells is absent. Incubation of CD38− LAK cells with cADPR (250 μM) stimulates cell migration. *, \(p < 0.05\); **, \(p < 0.005\). C, cGMP/PKG stimulates migration of LAK cells. Cells pretreated with Rp-8-pCPT-cGMPS (20 μM) for 30 min were incubated with IL8 (10 pM). For the determination of cGMPS-mediated cell migration, the cell-permeable cGMP analog, 8-pCPT-cGMP (1 mM), was used. **, \(p < 0.005\).

The treatment of various agents shows that cGMP increases [Ca\(^{2+}\)]\(_i\) [Ca\(^{2+}\)] in LAK cells, including T cells, is not activated unspecifically by increased [Ca\(^{2+}\)]\(_i\). Our results also indicate that cADPR induces Ca\(^{2+}\) influx in LAK cells. The depletion of extracellular Ca\(^{2+}\) using EGTA generates the initial Ca\(^{2+}\) rise but not the sustained Ca\(^{2+}\) signal in response to IL8. CD38/cADPR-mediated Ca\(^{2+}\) entry has been observed with neutrophils (11) and intact human T cells (36). A recent study has reported that type 3 RyR plays an essential role in the sustained Ca\(^{2+}\) response in T cells (37). Although the molecular mechanism by which cADPR-mediated sustained Ca\(^{2+}\) rise remains to be clarified, our results strongly suggest that cADPR produced by CD38 is a Ca\(^{2+}\)-mobilizing second messenger and mediates the sustained phase of Ca\(^{2+}\) signal by means of Ca\(^{2+}\) influx in LAK cells.

Studies have indicated that cAMP activates the production of cADPR in artery smooth muscle cell (13) and that cGMP is the activator of A. californica ADPR-cyclase (17). Our results suggest that the activation of CD38 expressed in LAK cells is mediated by PKG activation via cGMP generated by IL8 signaling. Thus, the treatment of various agents shows that cGMP increases [cADPR], in LAK cells. Consistent with these results, cGMP induces the sustained rise of [Ca\(^{2+}\)]\(_i\), and a PKG blocker, Rp-8-pCPT-cGMPS, abolishes the sustained Ca\(^{2+}\) signal in response to IL8. CD38/cADPR-mediated Ca\(^{2+}\) entry has been observed with neutrophils (11) and intact human T cells (36). A recent study has reported that type 3 RyR plays an essential role in the sustained Ca\(^{2+}\) response in T cells (37). Although the molecular mechanism by which cADPR-mediated sustained Ca\(^{2+}\) rise remains to be clarified, our results strongly suggest that cADPR produced by CD38 is a Ca\(^{2+}\)-mobilizing second messenger and mediates the sustained phase of Ca\(^{2+}\) signal by means of Ca\(^{2+}\) influx in LAK cells.

Our results have indicated that CD38/cADPR plays a critical role in the IL8-mediated migration of LAK cells. Thus, IL8-mediated cell migration is blocked by an antagonistic cADPR analog, 8-Br-cADPR, and by inactivation of CD38 with β-NAD\(^{+}\). Moreover, cADPR alone is able to induce LAK cell migration. Consistent with the observations that cGMP/PKG
signaling activates CD38, the pretreatment of the PKG inhibitor blocks IL8-mediated migration of LAK cells. We have also found that IL8-induced LAK cell migration is strictly controlled by Ca\(^{2+}\) influx; in the absence of extracellular Ca\(^{2+}\), IL8-induced migration of LAK cells is not observed, and nonphysiological increase of [Ca\(^{2+}\)]\(_i\) does not induce cell migration. A previous study has also indicated that dendritic cell trafficking depends on Ca\(^{2+}\) influx induced by CD38/cADPR (38). Studies have demonstrated that the biological role of CD38/cADPR in LAK cells as well as natural killer cells isolated is to trigger lytic and secretory responses (27). This cytotoxic role of CD38 has been observed by direct ligation of CD38 with an agonist antibody IB4 (39). Taken together, CD38 plays important roles in migration and cytotoxicity of LAK cells, including natural killer cells. The present study shows the regulation-signaling pathway of CD38 through the sequential coupling of IL8R, Ca\(^{2+}\) and cGMP/PKG and describes the biological role of CD38 in this signaling pathway, which is involved in IL8-induced cell migration through the generation of cADPR/sustained Ca\(^{2+}\) elevation.

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Activation of CD38 by Interleukin-8 Signaling Regulates Intracellular Ca$^{2+}$ Level and Motility of Lymphokine-activated Killer Cells

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