Exposure to TARC alters β2-adrenergic receptor signaling in human peripheral blood T lymphocytes

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Heijink, Irene H., Edo Vellenga, Jaap Oostendorp, Jan G. R. de Monchy, Dirkje S. Postma, and Henk F. Kauffman. Exposure to TARC alters β2-adrenergic receptor signaling in human peripheral blood T lymphocytes. Am J Physiol Lung Cell Mol Physiol 289: L53–L59, 2005. First published March 4, 2005; doi:10.1152/ajplung.00357.2004.—The β2-adrenergic receptor (β2-AR) negatively regulates T cell activity through the activation of the Gs/adenylyl cyclase/cAMP pathway. β2-AR desensitization, which can be induced by its phosphorylation, may have important consequences for the regulation of T cell function in asthma. In the present study we demonstrate that the C-C chemokine thymus and activation-regulated chemokine (TARC) impairs the ability of β2-agonist fenoterol to activate the cAMP downstream effector cAMP-responsive element binding protein (CREB) in freshly isolated human T cells. The TARC-induced activation of Src kinases resulted in membrane translocation of both G protein-coupled receptor kinase (GRK) 2 and β-arrestin. Moreover, TARC was able to induce Src-dependent serine phosphorylation of the β2-AR as well as its association with GRK2 and β-arrestin. Finally, in contrast to CREB, phosphorylation of Src and extracellular signal-regulated kinase was enhanced by fenoterol upon TARC pretreatment. In summary, we show for the first time that TARC exposure impairs β2-AR function in T cells. Our data suggest that this is mediated by Src-dependent activation of GRK2, resulting in receptor phosphorylation, binding to β-arrestin, and a switch from cAMP-dependent signaling to activation of the MAPK pathway. We propose that aberrant T cell control in the presence of endogenous β-agonists promotes T cell-mediated inflammation in asthma.

The β2-adrenergic receptor (β2-AR) is expressed on a variety of cell types, including T lymphocytes, and belongs to the family of cell surface receptors referred to as G protein-coupled receptors (GPCR). Upon binding of β2-agonists (e.g., epinephrine) to their cognate receptor, the β2-AR associates with the Gs protein, which triggers adenyl cyclase (AC) activity and subsequently results in the formation of intracellular cAMP. The cAMP-dependent pathway is widely known as a negative regulator of T cell activity. Upon prolonged or repeated agonist stimulation, uncoupling of the β2-AR from the AC/cAMP system can be induced. This desensitization is regulated by receptor phosphorylation and subsequent receptor internalization, which proceeds slower than phosphorylation. Internalization is not strictly required for β2-AR desensitization and is thought to be an early step in β2-AR downregulation and/or resensitization (25, 33, 41). Phosphorylation of the β2-AR can be induced by several kinases. Most described candidates are the G-coupled receptor kinases (GRK) and protein kinase A (PKA), which are activated upon stimulation of the β2-AR (13). β2-AR phosphorylation mediated by GRK2 and GRK3, also called βARK1 and βARK2, creates a binding site for β-arrestin (3, 13, 17, 23), which inhibits β2-AR function by preventing association with the Gs protein (17). Furthermore, β-arrestin has been described to recruit c-Src to the receptor, thereby linking the receptor to Ras/Raf/extracellular signal-regulated kinase (ERK) activation (24). In addition to βARK and PKA, various heterologous stimuli (e.g., growth factors and cytokines) have been described to phosphorylate the β2-AR, for example by the activation of PKC, tyrosine kinases, or phosphatidylinositol 3-(PI3-)kinase (25, 28, 12, 9).

Because the β2-AR system functions to suppress T cell-mediated responses, β2-AR desensitization may have important implications for the pathophysiology of atopic asthma. Impaired function of the β2-AR system has been observed in peripheral blood lymphocytes during allergen-induced inflammatory reactions in asthma (7, 8, 26). The origin of this β2-adrenergic dysfunction is still largely unclear; however, it is unlikely to be caused by enhanced endogenous β2-agonist levels (21). It has been proposed that the release of proinflammatory mediators from the lung into the circulation is responsible for the β2-AR dysfunction in peripheral lymphocytes in asthma (27). In this respect, chemokines may be of interest, since the release of certain chemokines [e.g., C-C chemokine thymus and activation-regulated chemokine (TARC)] is thought to play a crucial role in airway inflammation in asthma. There is growing evidence that TARC plays an important role in the recruitment of T helper (Th) 2 cells to the lung tissue and that TARC is released upon allergen challenge in asthma (4, 6, 18, 22, 29, 34), whereas the role of other Th2-directing chemokines appears less important (4, 6, 29, 34). Most chemokine receptors are linked to the Gs protein. Activation of Gs proteins can induce Rho GTPases, PI3-kinase, and Src-related sevenless (SOS)-dependent signaling, leading to changes in the cytoskeleton and cellular responses required for directional migration (14). Additionally, Gs activity is known to counterregulate the function of the Gs protein and has been implicated in β2-AR dysfunction (39). Moreover, chemokines have been demonstrated to induce activation of GRKs and β-arrestin, which may be critical for cytoskeletal reorganization and migration (5, 11, 37). We hypothesized that this may not only induce cytoskeletal
changes and homologous desensitization of chemokine receptors, but also β2-AR desensitization. To investigate this, we analyzed the effects of TARC on β2-AR signaling in freshly isolated human T cells.

**MATERIALS AND METHODS**

*Isolation of the T cells.* Peripheral blood mononuclear cells from healthy volunteer platelet donors, of whom for ethical reasons no further information was available, were isolated by Ficoll-Hypaque (Lymphoprep; Nycomed, Oslo, Norway) density-gradient centrifugation. The Human Subject Review Board of the University of Groningen has approved the protocol. T cells were isolated by rosetting with 2-aminomethylisothiouronium bromide-treated sheep red blood cells (SRBC). The SRBC were lysed by 155 mmol/l NH4Cl, 10 mmol/l KHCO3, and 0.1 mmol/l EDTA. The remaining cell preparations contained >98% T cells, as assessed by flow cytometric analysis after staining with α-CD2 (Becton Dickinson, Eredogem-Aalst, Belgium). T cells were incubated overnight at 37°C in RPMI 1640 (BioWhittaker, Verviers, Belgium) containing 0.5–1% fetal calf serum (FCS; Hyclone, Logan, UT), supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin.

*Immunoassay by Western blotting.* The phosphorylation of cAMP-responsive element binding protein (CREB), ERK, and Src family kinases was analyzed by Western blotting. T cells were pretreated with 10 μM PP2 (Calbiochem, Omnilabo International, Breda, The Netherlands) or 100 ng/ml pertussis toxin (PTX, Calbiochem, Omnilabo International) for 1 and 3 h, respectively, and subsequently incubated with or without TARC (250 pg/ml to 100 ng/ml) for 4–6 h. Next, cells were stimulated with 10 μM fenoterol (Sigma, St. Louis, MO) or 10 mM NaF (Sigma) for 0, 10, 30, 60, 90, or 180 min. Cells were harvested and spun down at maximum speed for 30 s. Total cell lysates were obtained by resuspension of the pellets in 1 × sample buffer (containing 2% SDS, 10% glycerol, 2% 2-mercaptoethanol, 60 mM Tris-HCl pH 6.8, and bromophenol blue) and boiling for 5 min. Samples were loaded on an SDS 10% PAGE gel (acrylamide-bisacrylamide 173:1) and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Hitchin, Herts, UK). Phospho-CREB, phospho-ERK, phospho-Src (New England Biolabs, Hitchin, Herts, UK), pan-ERK or actin (Santa Cruz Biotechnology) and incubated rotating at 4°C for 3 h. Protein bands were visualized using the gel scan program ImageMaster (Pharmacia, Uppsala, Sweden) and normalized for total protein levels.

*Immunodetection by Western blotting.* The phosphorylation of CREB, cAMP binding protein (CBP), phospho-ERK, phospho-Src (New England Biolabs, Hitchin, Herts, UK), and phospho-arrestin-2, IL-1R, and tubulin (Santa Cruz Biotechnology) using ECL according to the manufacturer’s guidelines.

*Immunoprecipitation.* T cells (10 × 10^6) were pretreated with 10 μM fenoterol (Sigma) or 100 ng/ml pertussis toxin (PTX, Calbiochem, Omnilabo International) for 1 h. Cells were harvested, washed with ice-cold PBS containing 1 mM sodium orthovanadate and 1 mM PMSF, and subsequently lysed in 500 μl of lysis buffer (50 mM Tris, pH 7.4, 10% glycerol, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 1 mM DTT, 1 μM pepstatin, Complete, 1 mM PMSF, and 1 mM sodium orthovanadate) for 10 min on ice. Cell lysates were clarified at 10,000 g for 15 min and pre-cleared with 30 μl of protein A Sepharose beads (in a 50% slurry) for 1 h at 4°C. After 1-min centrifugation at 300 g, the cell lysates were subjected to immunoprecipitation with antibodies specific for β2-AR, β-arrestin-2, GRK2-agarose conjugate (GRK2-AC, Santa Cruz Biotechnology) and incubated rotating at 4°C for 3 h or overnight. In case of immunoprecipitation with β2-AR and β-arrestin, protein A Sepharose beads were added to each sample and incubated rotating overnight to bind to the primary antibodies. Next, immune complexes were washed three times with lysis buffer. The precipitates were subjected to SDS 10% PAGE, immunoblotted on PVDF membrane, and immunocomplexes were detected by ECL, which was performed according to the manufacturer’s guidelines. The phosphoserine antibody was purchased from Zymed Laboratories (SANBIO, Uden, The Netherlands).

*Statistical analyses.* We used the Wilcoxon signed-rank test for paired observations in case of the cAMP measurements. The Student’s t-test for paired observations was used in case of protein levels or measurements of T cell migration. Data are expressed as means ± SE. Statistical significance of the secretion data was set at P < 0.05.

**RESULTS**

TARC preincubation reduces the ability of β2-agonist fenoterol to activate CREB. We first examined whether TARC is able to induce alterations in β2-AR signaling by studying the effect on cAMP downstream effector CREB in freshly isolated T cells. By flow cytometry we observed that 18.5 ± 6% of the peripheral T cells from healthy donors expresses the TARC...
receptor CCR4 (n = 4, data not shown). In freshly isolated T cells from healthy donors, incubation with fenoterol (10 μM) resulted in a clear induction of CREB phosphorylation. This was observed after just 10 min of stimulation, with a maximal response (fivefold induction) between 60 and 90 min (Fig. 1A). Incubation with TARC alone for 60 min (data not shown) or 6 h (Fig. 1B) had no effect on CREB phosphorylation. When T cells were preincubated with TARC for 6 h and subsequently stimulated with fenoterol for 60 min, the ability of fenoterol to induce CREB phosphorylation was markedly reduced (approximately twofold, Fig. 1B). This effect of TARC was also observed at a concentration of 250 pg/ml. At 50 pg/ml, the suppressive effect of TARC could no longer be observed (Fig. 1C).

The ability of β2-agonist fenoterol to induce cAMP formation. To test whether TARC indeed reduces β2-AR-coupled AC activity, we studied intracellular cAMP formation by β2-AR stimulation upon TARC pretreatment. In freshly isolated T cells, we observed that 10 μM fenoterol induced a significant (approximately threefold) increase in intracellular cAMP accumulation after 10 min of stimulation (Fig. 1E). When T cells were first incubated with TARC for 6 h and then stimulated with fenoterol, cAMP accumulation was significantly reduced (by ~25%; P < 0.05, n = 6), whereas TARC preincubation had no significant effect on basal cAMP levels (Fig. 1E). This indicates that TARC preincubation reduces β2-AR coupling to the Gs/AC/cAMP pathway.

Immunoblotting and RT-PCR studies demonstrated no change in β2-AR expression and β2-AR mRNA levels, indicating that TARC does not alter β2-AR function by degradation or downregulation of receptor expression (data not shown).

**TARC induces Gi activation and Src kinase activity.** To further identify the mechanism of TARC-induced desensitization of the β2-AR, we investigated the pathways induced by TARC stimulation. Most chemokine receptors are coupled to Gi proteins, leading to Gs/AC/cAMP pathway. The TARC-induced Src phosphorylation could be blocked by treatment with PTX (Fig. 3B). In accordance, PTX and PP2 pretreatment blocked the TARC-induced phosphorylation of ERK (data not shown).

**Activity of Src family kinases is critically involved in TARC-induced β2-adrenergic dysfunction.** Next, we studied whether or not Src kinase activation is critical for the suppressive effect of TARC on fenoterol-induced CREB phosphorylation. To establish the role of Src kinases in this process, T cells were preincubated with PP2, followed by exposure to TARC. As demonstrated in Fig. 3C, the fenoterol-induced phosphoryla-
Interestingly, incubation with TARC triggered the kinase activity responsible for the membrane translocation of GRK2, which was inhibited by both PP2 (Fig. 4) and the selective Src inhibitor PTX for 1 and 3 h, respectively. Migration was induced by the presence of 100 ng/ml TARC. T cell migration is expressed as percentage of the total amount of T cells added to the upper well. T cells were incubated with or without Src inhibitor PP2 or Gi inhibitor pertussis toxin (PTX) for 1 and 3 h, respectively. Migration was induced by the presence of 100 ng/ml TARC. T cell migration is expressed as percentage of the total amount of T cells added to the upper well (B). Experiments were carried out in duplicate with T cells from 4 healthy donors, and means ± SE are shown.

TARC exposure (data not shown) and remained for at least up to 4 h (Fig. 4). The effect of TARC on GRK2 location was membrane translocation of GRK2 was already seen after 1 h of TARC exposure, and tubulin was used as cytosol marker. The increased membrane fraction (Fig. 4). As control for the purity of the subcellular fractions, IL-1R was used as membrane marker, and tubulin was used as cytosol marker. The increased membrane translocation of GRK2 was already seen after 1 h of TARC exposure (data not shown) and remained for at least up to 4 h (Fig. 4). The effect of TARC on GRK2 location was inhibited by both PP2 (Fig. 4) and the selective Src inhibitor SU-6656 (data not shown), indicating that TARC-induced Src kinase activity is responsible for the membrane translocation of GRK2. Interestingly, incubation with TARC triggered the membrane translocation of β-arrestin as well (Fig. 4), which was again blocked by PP2 pretreatment. This membrane translocation of GRK2 and β-arrestin may be involved in the TARC-induced effect on β2-AR function.

TARC induces Src kinase-dependent membrane translocation of GRK2 and β-arrestin. It is conceivable that Src kinase activity is involved in β2-AR desensitization, since Src kinases may phosphorylate and subsequently activate GRK2 (10, 33). Therefore, we tested whether TARC was able to induce Src-dependent activation and membrane translocation of GRK2. In resting T cells, GRK2 was localized in both cytosol and membrane fractions. TARC exposure reduced the expression of GRK2 in the cytoplasm and increased the expression in the membrane fraction (Fig. 4). As control for the purity of the subcellular fractions, IL-1R was used as membrane marker, and tubulin was used as cytosol marker. The increased membrane translocation of GRK2 was already seen after 1 h of TARC exposure (data not shown) and remained for at least up to 4 h (Fig. 4). The effect of TARC on GRK2 location was inhibited by both PP2 (Fig. 4) and the selective Src inhibitor SU-6656 (data not shown), indicating that TARC-induced Src kinase activity is responsible for the membrane translocation of GRK2. Interestingly, incubation with TARC triggered the

TARC induces Gi-dependent activation of the Src kinases and this is involved in the suppression effect on β2-adrenergic receptor (AR) function. Freshly isolated T cells were stimulated with TARC (100 ng/ml) for 5, 15, and 30 min. One hour before stimulation, PP2 was added. Total cell lysates were prepared and phosphorylated (p)-Src was detected by Western blotting (n = 3) (A). T cells were stimulated with TARC (100 ng/ml) for 5 min. Before stimulation, PTX was added. Total cell lysates were prepared. p-Src was detected by Western blotting (n = 5) (B). Freshly isolated T cells were preincubated with 100 ng/ml TARC for 6 h. Before TARC incubation, PP2 was added. Next, T cells were stimulated with fenoterol for 60 min. Total cell lysates were prepared, and p-CREB was detected by Western blotting (n = 4) (C). p-Src and p-CREB are depicted in the top panels, and total ERK levels are shown in the bottom panels (marked by arrows).

TARC exposure induces Src kinase-dependent membrane translocation of G-coupled receptor kinase (GRK) 2 and β-arrestin (Fig. 4). The expression of β-arrestin and GRK2 in cytosol and membrane fractions was detected by Western blotting and is indicated by arrows. IL-1R and tubulin indicate purity of the membrane and cytosolic fractionation and were used to establish equal loading. A representative blot of 4 independent experiments is shown.
TARC exposure induces serine phosphorylation of the β2-AR and association to GRK2 and β-arrestin. Next, we questioned whether the effects of TARC as described above lead to alterations in the phosphorylation status of the β2-AR. To this end, we determined the phosphoserine content of the β2-AR after immunoprecipitation. As demonstrated in Fig. 5A, the levels of serine-phosphorylated β2-AR were increased by ~1.5-fold when T cells were exposed to TARC (3 h), which was observed in absence of a β2-agonist. In addition, we studied the association of GRK2 and β2-AR by coimmunoprecipitation. As depicted in Fig. 5B, the association between β2-AR and GRK2 was markedly increased when T cells were exposed to TARC, supporting a role for GRK2 in the β2-AR serine phosphorylation. Furthermore, by coimmunoprecipitation of β-arrestin and β2-AR we observed that TARC induces recruitment of β-arrestin to the β2-AR (Fig. 5C). In accordance to the effects as described in the previous paragraphs, the TARC-induced β2-AR phosphorylation and recruitment of βARK and β-arrestin were blocked by PP2 (Fig. 5, A–C). Together, these data suggest that the activation of Src family kinases by TARC induces GRK2-mediated phosphorylation of the β2-AR and subsequent complex formation with β-arrestin in an agonist-independent manner.

Finally, we tested whether the TARC-induced recruitment of β-arrestin to the β2-AR results in a switch to activation of the Src/MAPK pathway. To this end, we studied whether or not fenoterol was able to induce phosphorylation of Src family kinases and ERK in T cells that were exposed to TARC (3 h). In resting T cells, basal Src kinase activity was inhibited by fenoterol after 10 and 60 min of incubation (Fig. 6A). In contrast, fenoterol induced a modest increase in Src phosphorylation when T cells were first exposed to TARC. Similarly, fenoterol enhanced ERK phosphorylation after preincubation with TARC (Fig. 6B). This indicates that TARC exposure switches β2-AR function from activation of the Gs/AC/cAMP system, which negatively regulates the Src/SOS/MAPK pathway, to activation of the Src/MAPK pathway. Thus, after TARC exposure, T cell activity may be enhanced instead of inhibited by the β2-AR system.

**DISCUSSION**

The β2-AR is linked to the Gs protein and AC/cAMP activation, thereby providing negative feedback control over various proinflammatory cells. Prolonged or repeated stimulation of the β-agonist as well as exposure to various heterologous stimuli (e.g., growth factors and cytokines) may induce desensitization of the β2-AR, resulting in impaired activation of the cAMP-dependent pathway (12, 35). We have previously demonstrated that allergen exposure in asthma reduces β2-AR function for 40–50% in the total pool of peripheral blood lymphocytes, resulting in a loss of control over Th2-like cytokines (16, 26, 27). This loss of β2-AR function is thought to be the result of allergen-induced release of proinflammatory mediators and is not observed during stable asthma (27). However, the extrinsic factor(s) responsible for the β2-AR desensitization has remained unidentified. In the present study we demonstrate for the first time that TARC-induced activation of the C-C chemokine receptor CCR4, which is expressed on ~50% of the memory Th cells (19), results in desensitization of the β2-AR in peripheral blood T lymphocytes. Our results demonstrate that TARC induces Src-dependent membrane
translocation of GRK2 and β-arrrestin, resulting in β2-AR phosphorylation and defective cAMP-dependent signaling. This observation may have important implications for T cell-mediated immune responses, including airway inflammation in asthma, since T cell activity is normally controlled by the β2-AR/AC system. Indeed, we have previously demonstrated that Th1 and Th2 cytokine expression in α-CD3/α-CD28-stimulated, freshly isolated human T cells is inhibited by the β2-agonist fenoterol (15). Interestingly, we have recently observed that the allergen-induced loss of β2-AR control over Th2 cytokines could be mimicked by TARC exposure in T cells from healthy donors (16). In addition, we observed that TARC has a similar effect in stable asthma (data not shown). These data indicate that TARC exposure may induce important alterations in the regulation of T cell function.

In this report we demonstrate that TARC exposure reduces AC activity and cAMP-dependent signaling upon stimulation with the β2-agonist fenoterol in peripheral T cells. The effect of TARC on cAMP accumulation was not as pronounced as the effect on CREB phosphorylation. Recently, it has been shown that cAMP accumulation and CREB phosphorylation do not always correlate. Partial β-agonists that hardly increased intracellular cAMP were shown to induce an increase in CREB phosphorylation (2). This may be explained by the fact that it is not the total quantity of cAMP that is responsible for CREB activation, but cAMP’s sustained turnover (2). cAMP turnover can be regulated by β-arrrestin, since β-arrrestin has been shown to target cAMP degradation to the receptor by its binding to phosphodiesterase (PDE) 4 (30). Thus TARC might affect cAMP accumulation by the recruitment of β-arrrestin/PDE4 to the β2-AR, thereby simultaneously reducing cAMP accumulation by desensitization and increasing the rate of cAMP degradation at the membrane. Because we used IBMX in the cAMP assay to prevent cAMP degradation and to obtain sufficient high cAMP accumulation, the possible additional effect of PDE4-mediated degradation could not be observed.

The results with NaF indicate that the effect of TARC on β2-adrenergic signaling is most likely mediated upstream from the G1/AC system at receptor level and not by direct inhibition of the AC system. Furthermore, our data suggest the involvement of G1-dependent activation of Src family kinases in reduced β2-AR function. We observed that TARC induces Src-dependent membrane translocation of GRK2 and β-arrrestin. Although Gβγ subunits are thought to be essential for the membrane anchoring of GRK2 (31), our results indicate that Src activation is also required for the TARC-induced membrane translocation of GRK2 and subsequent β2-AR phosphorylation. This is in accordance with the findings of Sarnago et al. (33), demonstrating that Src activation induces GRK2 phosphorylation and activation. In addition, the findings of Fan et al. (10) demonstrate that Src activation precedes GRK2-mediated receptor phosphorylation and binding to β-arrrestin. Src is known to associate with β-arrrestin upon β2-AR stimulation (24), but the role of Src kinases in β-arrrestin membrane translocation has to our knowledge not been described before. In the present study we show that Src kinase activation by TARC induces the membrane translocation and recruitment of β-arrrestin to the β2-AR. The membrane translocation of β-arrrestin may be a direct effect of Src or a consequence of β2-AR phosphorylation. Either way, receptor phosphorylation is required to induce binding of β-arrrestin to the receptor. Subsequently, β-arrrestin may promote the complex formation of β2-AR/Src (24), which might be responsible for the observed switch of β2-AR signaling to activation of the MAPK pathway. The positive β2-AR signaling observed after TARC exposure may enhance the activation profile of T cells. In accordance to the findings concerning β-arrrestin, we have recently been reported that β-arrrestin-2 is crucial for Th2 migration to the lung in a mouse model of asthma. β-Arrrestin-2 knockout mice did not accumulate T cells in the lung and had defective macrophage-derived chemokine-mediated T cell migration (40).

Our findings suggest that the activation of GRK2 and β-arrrestin by TARC does not facilitate agonist-induced β2-AR desensitization but induces β2-agonist-independent desensitization of the β2-AR. So far, it has been assumed that βARK mediates homologous desensitization of GPRCs, as GRK2 preferentially phosphorylates the agonist-occupied receptor. However, agonist-independent phosphorylation of the β2-AR has been demonstrated in other cell systems. For instance, insulin can induce agonist-independent phosphorylation of the β2-AR in A431 cells (9). In addition, it has recently been described in HEK-293 cells that EGF receptor internalization is triggered by β2-AR stimulation in the absence of EGF, in a GRK2- and β-arrrestin-dependent manner (20). These findings clearly illustrate that GRK2-mediated desensitization is not restricted to homologous desensitization. Furthermore, chemokines have been demonstrated to induce cross-desensitization of another GPCR, i.e., the μ-opioid receptor, yet the mechanism remained undefined (38). The chemokine-induced desensitization of the β2-AR may be an important mechanism in the amplification of inflammation, as it may result in enhanced T cell activity in presence of endogenous β2-agonists. In addition to TARC, we observed that the pretreatment with the chemokines monocyte chemoattractant protein-1, IL-8, and 10-kDa interferon-γ-inducible protein similarly reduced β2-AR signaling in T cells (data not shown), indicating that chemokines in general have an important role in the regulation of β2-AR function. Our findings may have implications for additional cell types, including mast cells, eosinophils, and smooth muscle cells, which are known to be under control of the β2-AR system and express various chemokine receptors as well.

In summary, we demonstrate that exposure of T cells to TARC alters the function of the β2-AR, resulting in impaired ability to activate the cAMP-dependent pathway and a switch to Src/MAPK activation. This may be mediated by the Src-dependent membrane translocation of GRK2, phosphorylation of the β2-AR, and recruitment of β-arrrestin and result in dysregulated and enhanced activity of T cells by circulating endogenous β2-agonists.

GRANTS
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