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Specific induction of cAMP in Langerhans cells by calcitonin gene-related peptide: Relevance to functional effects

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ABSTRACT **Epidermal Langerhans cells (LC) are associated anatomically with epidermal nerves, and a product of these nerves, calcitonin gene-related peptide (CGRP), inhibits the antigen-presenting capacity of LC and macrophages. As the CGRP receptor appears to be coupled to G_s α protein, which in turn activates adenylate cyclase, the ability of CGRP to induce cAMP in LC was examined and correlated with functional effects. LC were isolated from murine epidermal cells using antibodies on magnetic microspheres. Exposure to CGRP induced a significant increase in cAMP content, which could be inhibited by coculture with a truncated form of CGRP [CGRP-(8–37)] that is a specific competitive inhibitor of CGRP. Substance P and calcitonin failed to induce cAMP in LC. Although culture in CGRP reduced the ability of murine epidermal cells enriched for LC content to present pigeon cytochrome *c* to a responsive clone or to present antigen for elicitation of delayed-type hypersensitivity in immune mice, culture in forskolin had little or no effect on antigen presentation despite increased cAMP content of LC as much or more than that induced by CGRP. The effect of CGRP on antigen presentation in these systems could be blocked with CGRP-(8–37). CGRP inhibited the induction of B7-2 by lipopolysaccharide on peritoneal macrophages and a LC line, whereas calcitonin did not. CGRP induces specific accumulation of cAMP in LC and inhibits LC antigen-presenting function by a receptor-mediated event. However, the induction of cAMP by itself does not account for inhibition of antigen presentation. Suppression of the expression of B7-2 may be one mechanism by which CGRP inhibits antigen presentation.**

Langerhans cells (LC) are dendritic antigen-presenting cells in the skin that play a key role in the cutaneous immune system (1). We have recently discovered an anatomical association between LC and peripheral nerves and have hypothesized that the nervous system regulates the function of LC via release of neuron-derived factors, resulting in the modulation of cutaneous immune responses (2). In particular, calcitonin gene-related peptide (CGRP), a major neuropeptide of peripheral sensory nerves (3), was found on the surface of some epidermal LC, and CGRP inhibited the antigen-presenting function of LC in some assay systems (2). We aimed to elucidate this relationship further and to obtain direct evidence for the presence of specific receptors for CGRP on LC.

Many neuropeptides (4), as well as many neurotransmitters (5), transduce signals through specific membrane receptors that belong to the large family of G protein-coupled receptors. Two major second-messenger systems, the cAMP–protein kinase A pathway and the phosphatidylinositol–protein kinase C (PKC) pathway, are activated through G proteins, and several ion channels are also modulated by second-messenger systems coupled to G proteins (6). The CGRP receptor appears to be coupled to G_s α subunit, which in turn activates adenylate cyclase (7). Increases in cellular cAMP concentration and/or adenylate cyclase activity have been reported after stimulation of CGRP receptors on various targets (8–10). Therefore, measurement of the intracellular cAMP level in LC was used to demonstrate that an interaction between CGRP and putative CGRP receptors occurs in LC.

LC were isolated from murine epidermal cells (EC) (11, 12) and demonstrated specific intracellular cAMP formation as a rapid response after incubation with CGRP. This response was then compared with that of keratinocytes (KC) and macrophages (Mϕ). Using functional assay systems, we addressed the following issues: (*i*) whether this receptor-mediated response accounted for the inhibitory effect of CGRP on LC, and (*ii*) whether cAMP alone was responsible for this effect as the second messenger. Finally, we examined the effect of CGRP on induction of B7-2 expression as a possible mechanism of CGRP inhibitory activity on antigen presentation.

MATERIALS AND METHODS

Animals. Six- to 12-week-old female BALB/c (H-2^d), A/J (H-2^a), and (BALB/c × A/J) (CAF_1) (H-$2^{d/a}$) mice were obtained from The Jackson Laboratory.

Test Reagents. CGRP-I (rat), CGRP-(8–37) (human), substance P (SP) (rat), and vasoactive intestinal polypeptide (VIP) (rat) were purchased from Peninsula Laboratories, rat calcitonin (CT) was from Sigma, and forskolin (FK) was from Calbiochem.

Tumor Cell Line and Preparation of Tumor-Associated Antigens (TAA). The S1509a methylcholanthrene-induced spindle cell tumor line (H-2^a) was maintained at 37°C and 5% CO_2/95% air in RPMI 1640 medium/10% heat-inactivated fetal calf serum (Life Technologies, Gaithersburg, MD)/penicillin at 100 units/ml/streptomycin at 100 μg/ml/2 mM L-glutamine/0.1 mM nonessential amino acids/1 mM sodium pyruvate/10 mM Hepes (complete medium). A freeze–thaw lysate was used as a source of soluble TAA as described elsewhere (13).

T-Cell Clone. The pigeon cytochrome *c* (PCC)-specific, I-E^k-restricted Th1 clone A.E7 (14) was grown at 37°C and 5% CO_2/95% air in complete medium/50 μM 2-mercaptoethanol/amphotericin at 0.25 μg/ml/gentamicin at 50 μg/ml (A.E7 medium). Irradiated A/J spleen cells (5×10^5 cells) were mixed with 2×10^4 AE7 cells. Recombinant interleukin (IL) 2 (Biosource, Camarillo, CA) was added at 10 units/ml at 4 days of culture, and PCC (Sigma) was added to a final concentration of 50 μg/ml. The cells were passed over Lym-

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Abbreviations: LC, Langerhans cells; EC, murine epidermal cells; CGRP, calcitonin gene-related peptide; PKC, protein kinase C; KC, keratinocytes; SP, substance P; VIP, vasoactive intestinal polypeptide; PCC, pigeon cytochrome *c*; IL, interleukin; FK, forskolin; DTH, delayed-type hypersensitivity; TAA, tumor-associated antigens; LPS, lipopolysaccharide; Mϕ, macrophages; CT, calcitonin.

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pholyte M (density, 1.0875; Cederlane Laboratories) density gradients (300 × *g* for 20 min at room temperature) every 3 weeks for isolation and restimulation of A.E7 cells as above.

Peritoneal Mϕ Preparation. BALB/c mice were injected i.p. with 2.5 ml of 3% thioglycolate broth (Difco). Cells were harvested at 4 days and were 80–90% Mac-1 positive by flow cytometry.

LC Line. A dendritic cell line (XS52) that presents antigen and has many phenotypic characteristics of freshly harvested LC (15–17) was established from newborn BALB/c epidermis and propagated as described (15).

EC Preparation. EC were prepared, and Thy-1$^+$ cells were deleted as reported (13). For functional assays, dead cells were removed by treatment with 0.05% trypsin and DNase at 80 μg/ml (Sigma) in Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS) for 3 min at room temperature to yield LC content of 5–10% by I-A positivity. For cAMP measurement, to remove dead cells, cells were passed over Lympholyte M density gradients as above, and interface cells were harvested.

Isolation of LC from the EC Preparations. EC from BALB/c mice were incubated with mouse anti-mouse I-A^d (PharMingen) for 30 min on ice followed by incubation with goat anti-mouse IgG conjugated to magnetic microspheres (Dynabeads M-450; Dynal, Oslo) for 30 min on ice with continuous gentle shaking. The cells were then separated into two fractions by washing under a magnetic field, and those with attached magnetic beads were designated as LC, whereas those without were designated as KC.

Measurements of Intracellular cAMP Accumulation. For intracellular cAMP measurements, cells (EC without depletion of Thy-1$^+$ cells, LC, KC, or peritoneal Mϕ) were cultured in RPMI 1640 medium/bovine serum albumin at 1 mg/ml (Sigma)/1 mM isobutylmethylxanthine (Sigma) for 10 min at 37°C, in the absence or presence of various concentrations of test agents [CGRP-I, CGRP-I/CGRP-(8–37), CT, SP, VIP, and FK]. Incubations were terminated by centrifugation, aspiration of medium, and addition of 0.05 M Tris·EDTA buffer/4 mM EDTA, pH 7.5. Samples were heated for 5 min in boiling water and centrifuged; supernatants were then measured for cAMP content with a RIA kit (cAMP [^{3}H]radioassay system; Amersham).

Flow Cytometry. To determine the I-A^d positivity of LC and KC subpopulations after magnetic microsphere separation, fluorescein isothiocyanate-conjugated anti-mouse I-A^d (PharMingen) was used. I-A^d positivity was analyzed by FACScan (Becton Dickinson). To analyze B7-2 expression by activated peritoneal Mϕ, cells were incubated for 24 hr at 37°C with or without lipopolysaccharide (LPS) in the presence or absence of CGRP-I, CT, or FK. The gate for Mϕ was determined by staining with fluorescein isothiocyanate-conjugated anti-mouse Mac-1 (Caltag, South San Francisco, CA), and B7-2 positivity was determined by staining with fluorescein isothiocyanate-conjugated anti-mouse B7-2 (PharMingen). Negative control staining was done with irrelevant fluorescein isothiocyanate-conjugated immunoglobulin. To examine B7-2 expression by XS52 cells, cells were cultured without granulocyte/macrophage colony-stimulating factor (GM-CSF) for 48 hr. Then, they were cultured for 24 hr in medium containing GM-CSF at 50 units/ml and LPS at 0.1 μg/ml in the presence or absence of CGRP-I, CT, or FK before staining.

Immunization Protocol and Challenge to TAA. CAF_1 mice were immunized three times at weekly intervals by s.c. injection of 2 × 10^6 disrupted S1509a cells. Mice were challenged 1 week after the last immunization. EC were incubated for 3 hr at 37°C in medium alone, 100 nM CGRP-I, 100 nM CGRP-I/10 μM CGRP-(8–37), or 1 μM FK. EC were then washed and incubated in TAA from S1509a cells for 3 hr at 37°C. Cells were then washed extensively, and 5 × 10^5 cells were injected s.c. into the left hind footpad. A group of naive mice were challenged in the same way (negative control). Footpad swelling was measured after 24 hr as described (13).

Proliferation Assay of T-Cell Clone A.E7. A.E7 cells were purified by lysis of A/J feeder cells by incubation with anti-mouse I-A^k (PharMingen) at 1:50 for 30 min on ice, washing, and subsequent incubation in low-toxicity rabbit complement at 1:40 for 30 min at 37°C. Cells were then passed over Lympholyte M density gradients, and interface cells were harvested. EC from A/J mice were incubated for 5 hr at 37°C in medium alone, 100 nM CGRP-I, or 1 μM FK. PCC was added (50 μg/ml) for the last 3.5 hr. After incubation, cells were washed and counted; then 0.5–3 × 10^5 EC were mixed with A.E7 cells (2 × 10^4 cells per well) in 96-well flat plates in A.E7 medium/indomethacin at 1 μg/ml (Sigma). Cells were cultured for 72 hr with 1 μCi per well of [^{3}H]thymidine (1 Ci = 37 GBq; New England Nuclear) added for the last 20 hr and harvested; incorporation of [^{3}H]thymidine was assessed as described (2).

Statistical Analysis. Differences among groups were examined by Student's *t* test.

RESULTS

Isolation of LC from Murine EC Suspensions and cAMP Formation in Response to CGRP-I. Using immunomagnetic separation, we obtained cell populations that were 80–90% LC as determined by flow cytometry. The negatively selected population was <5% I-A^d positive (considered to be primarily KC). After 10 min of 100 nM CGRP-I incubation, the level of cAMP in LC was significantly elevated, whereas both fresh EC and the negatively selected KC responded only modestly (Fig. 1). CGRP-II, which is different at amino acid position 35, was as potent as CGRP-I in inducing cAMP in LC (data not shown).

Dose-Dependent Formation of cAMP and Inhibition by CGRP-I Antagonist CGRP-(8–37). CGRP-I induced intracellular cAMP in LC in a dose-dependent fashion (Fig. 2). KC also showed a dose-dependent increase, but the induction level remained low. In contrast, peritoneal Mϕ did not show a consistent dose-dependent response, and the level induced was lower than in KC at some points. To demonstrate that cAMP induction in LC is receptor-mediated, we examined the ability of the CGRP-I antagonist CGRP-(8–37), a truncated form of CGRP-I missing the first 7 amino acids, to prevent the CGRP-induced accumulation of cAMP in LC. CGRP-(8–37)

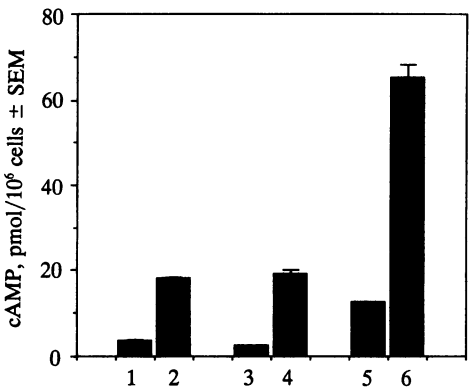

FIG. 1. Intracellular cAMP accumulation induced by CGRP in EC, KC, and LC. EC were generated from BALB/c mice and separated by immunomagnetic antibody techniques to yield LC (I-A$^+$) and KC (I-A$^-$). Cells were incubated for 10 min at 37°C in RPMI 1640 medium/bovine serum albumin at 1 mg/ml/1 mM isobutylmethylxanthine with the absence [unseparated EC (bar 1), KC (bar 3), LC (bar 5)] or presence [unseparated EC (bar 2), KC (bar 4), LC (bar 6)] of 100 nM CGRP-I. Incubations were terminated, and cAMP content was assessed. SEM represents the results of three experiments.

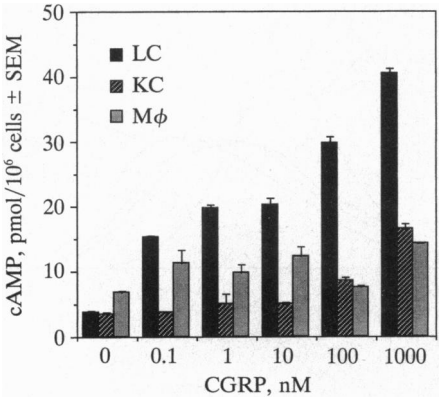

FIG. 2. Dose–response relationship of cAMP accumulation induced by CGRP in LC, KC, and Mϕ. LC, KC, and peritoneal Mϕ were generated from BALB/c mice and were incubated for 10 min at 37°C in medium alone or in 0.1 nM, 1 nM, 10 nM, 100 nM, or 1 μM CGRP-I. SEM represents the results of three experiments.

in excess during the 10-min incubation antagonized the effect of CGRP-I dose-dependently (Fig. 3). CGRP-(8–37) did not block the effect of FK on cAMP accumulation (data not shown).

Comparison of the Effect of cAMP Induction by CGRP-I with Other Peptides. Other neuropeptides reported to be present in the epidermis were tested at 100 nM (Fig. 4). In LC, VIP induced cAMP accumulation comparable to that seen with CGRP. In KC, VIP showed only a moderate induction. SP and CT failed to induce cAMP formation in either LC or KC. FK at 1 μM served as a positive control and induced relatively large quantities of cAMP in both LC and KC—2.9 times as much in LC and 2.7 times as much in KC as elicited by 100 nM of CGRP-I.

Specificity of the Effect of CGRP-I on Elicitation of Delayed-Type Hypersensitivity (DTH) by EC. EC were treated with TAA and used to challenge immunized mice for a DTH reaction. DTH elicited was significantly inhibited by incubation of EC with 100 nM CGRP-I before treating with TAA (Fig. 5). The addition of CGRP-(8–37) at 100-fold excess during CGRP incubation completely blocked this effect (Fig. 5), indicating that the effect is receptor-mediated. One μM FK, which induces more cAMP than 100 nM CGRP-I, failed to inhibit the elicitation of DTH (Fig. 6).

Specificity of the Effect of CGRP-I on EC Presentation of PCC to A.E7 Cells. Treatment with CGRP significantly inhibited EC presentation of PCC to A.E7 cells (Fig. 7). FK at 1 μM failed to significantly inhibit antigen presentation, although a trend toward inhibition was observed at some stimulator to responder ratios in some experiments (Fig. 7). Additional experiments demonstrated that inhibition of the antigen-presenting capability of EC by CGRP-I could be completely blocked by CGRP-(8–37) added at 100-fold excess and that incubation of EC in 100 nM CT failed to alter EC presentation of PCC to A.E7 cells (data not shown).

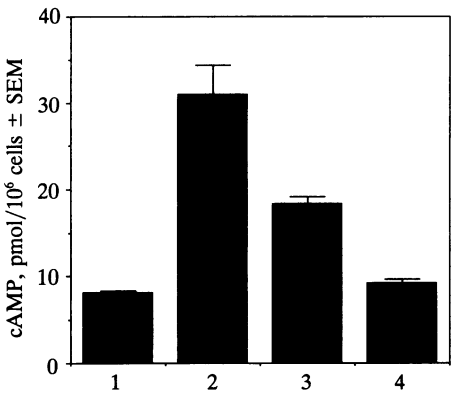

FIG. 3. cAMP formation by CGRP is blocked by coincubation with the CGRP antagonist CGRP-(8–37). LC were generated from BALB/c mice and incubated for 10 min at 37°C in medium alone (bar 1), 100 nM CGRP-I (bar 2), 100 nM CGRP-I/1 μM CGRP-(8–37) (bar 3), or 100 nM CGRP-I/10 μM CGRP-(8–37) (bar 4). SEM represents the results of three experiments.

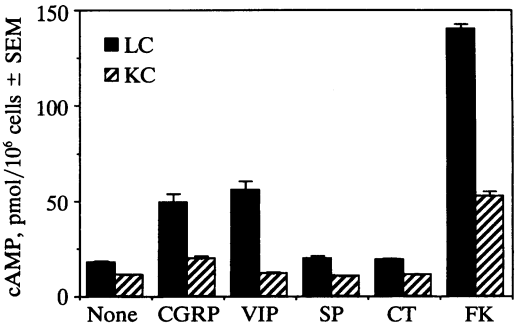

FIG. 4. Comparison of the effect of induction of cAMP by CGRP with other peptides. LC and KC were generated from BALB/c mice and then incubated for 10 min at 37°C in medium alone, or 100 nM CGRP-I, 100 nM VIP, 100 nM SP, 100 nM CT, or 1 μM FK. SEM represents the results of three experiments.

CGRP-I Modulation of B7-2 Expression by Activated Peritoneal Mϕ and XS52 Cells. Thioglycolate-elicited Mϕ were incubated in LPS with or without test agents for 24 hr at 37°C, and induction of B7-2 was examined by flow cytometry (Fig. 8*A*). Induction of B7-2 was suppressed by CGRP-I, whereas neither FK nor CT was inhibitory under these conditions (data not shown). Little or no change in Ia expression was observed with these conditions, and B7-2 expression remained unchanged by FK in the absence of LPS stimulation (data not shown). Induction of B7-2 expression by XS52 cells was also suppressed by CGRP-I (Fig. 8*B*) but not by CT or FK (data not shown).

DISCUSSION

CGRP is a 37-amino acid peptide widely distributed in the central and peripheral nervous systems (3) that has potent

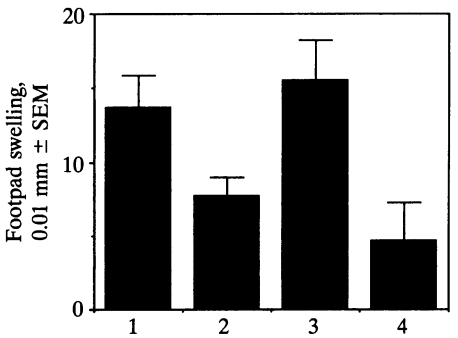

FIG. 5. Inhibition of EC presentation of TAA for elicitation of DTH by CGRP is blocked by coincubation in CGRP-(8–37). EC were generated from CAF_1 mice and enriched for LC by deletion of Thy-1$^+$ cells. EC were then incubated for 3 hr at 37°C in either medium alone (bar 1), 100 nM CGRP-I (bar 2), or 100 nM CGRP-I/10 μM CGRP-(8–37) (bar 3). EC from each group were then pulsed with soluble TAA derived from S1509a and washed extensively. EC from each group were then injected s.c. into the left hind footpad of CAF_1 mice previously immunized to S1509a cells. A group of naive mice were challenged with TAA-treated EC not exposed to peptides (negative control) (bar 4). Footpad swelling was assessed after 24 hr as a measure of DTH response. For all groups $n = 6$. Bar 1 vs. 2, $P < 0.04$; bar 1 vs. 4, $P < 0.03$; bar 1 vs. 3, not significant.

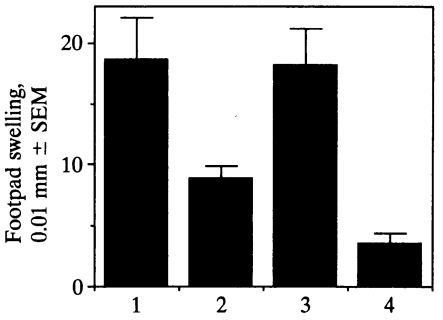

FIG. 6. FK does not inhibit EC presentation of TAA for elicitation of DTH. EC were generated from CAF_1 mice and enriched for LC. EC were then incubated for 3 hr at 37°C in either medium alone (bar 1), 100 nM CGRP-I (bar 2), or 1 μM FK (bar 3). EC from each group were then treated with soluble TAA and injected s.c. into the left hind footpad of CAF_1 mice previously immunized to S1509a cells. A group of naive mice were challenged with TAA-treated EC not exposed to peptides (negative control) (bar 4). Footpad swelling was assessed after 24 hr as a measure of DTH response. For all groups $n = 6$. Bar 1 vs. 2, $P < 0.03$; bar 1 vs. 4, $P < 0.004$; bar 1 vs. 3, not significant.

vasodilating properties (18). CGRP also has modulatory effects on immunocompetent cells such as T cells (10), B cells (19), and Mϕ (20). CGRP also inhibits the antigen-presenting capability of LC (2).

To demonstrate an interaction between CGRP and putative CGRP receptors on LC, the level of a second messenger was assessed in this study. The CGRP receptor appears to be a G protein-linked receptor (4, 5), which activates adenylate cyclase leading to cAMP formation (8–10). Immunomagnetic separation (11, 12) was used to obtain a highly purified population of LC.

Increases in cAMP were observed in a dose-dependent manner after CGRP administration to purified LC, suggesting that LC have functional receptors for CGRP coupled to G_s. CGRP-(8–37) blocked this elevation in a dose-dependent manner, indicating specific binding of CGRP to its receptor. Moreover, CT, which is encoded by the same gene as CGRP

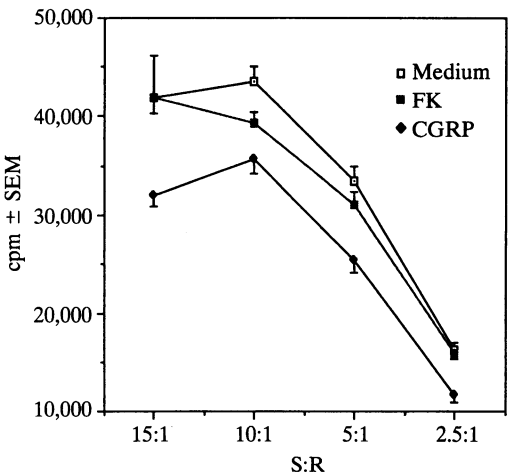

FIG. 7. Effects of CGRP and FK on EC presentation of PCC to A.E7 cells. A/J EC were generated and enriched for LC as described. EC were then incubated for 5 hr at 37°C in either medium alone, 100 nM CGRP-I, or 1 μM FK. PCC was added at 50 μg/ml for the last 3.5 hr. The cells were then washed and mixed with 2×10^4 A.E7 cells per well in 96-well flat-bottom plates. Incubation was continued for 72 hr with 1 μCi per well of [^{3}H]thymidine added for the last 20 hr, and incorporation of radioactivity was assessed. SEM represents six wells. When EC were mixed with A.E7 cells without exposure to antigen, cpm were <1000 at all stimulator/responder (S:R) ratios examined.

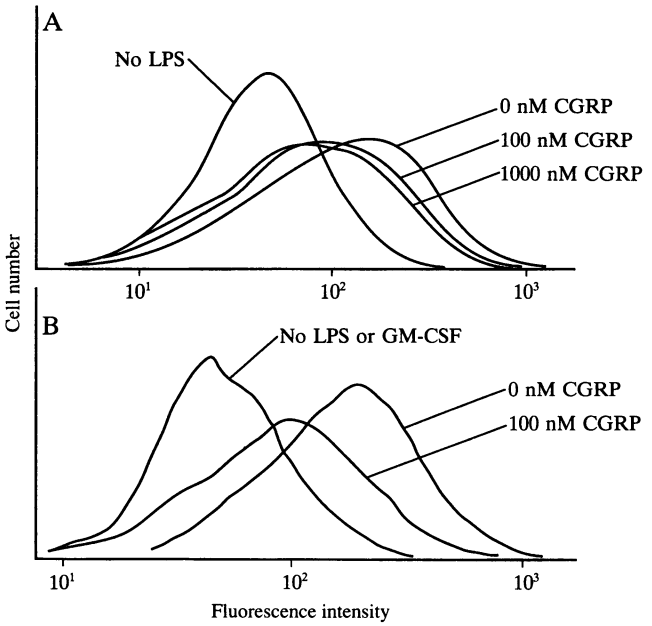

FIG. 8. Inhibition of the induction of B7-2 expression by CGRP in peritoneal Mϕ and XS52 cells. (*A*) Thioglycolate-elicited peritoneal Mϕ were prepared and incubated for 24 hr at 37°C in medium containing or not containing LPS at 0.1 μg/ml with or without CGRP-I. Cells were then washed and examined by flow cytometry for B7-2 expression. Cells exposed to LPS but kept at 4°C demonstrated less expression of B7-2 than the no-LPS group with or without the presence of CGRP. (*B*) XS52 cells were incubated for 24 hr at 37°C in medium containing or not containing LPS at 0.1 μg/ml and granulocyte/macrophage colony-stimulating factor (GM-CSF) at 50 units/ml in the presence or absence of CGRP-I.

but produced by an alternative splicing (18), did not induce cAMP.

CGRP induced higher levels of cAMP in LC than in KC under the same conditions. cAMP formation by CGRP in cultured human KC has been reported (4). We found that, in the absence of CGRP but in the presence of isobutylmethylxanthine, the basal level of cAMP is usually higher in LC than KC. The level of cAMP after FK stimulation was also higher in LC compared to KC. Hypothetically, this responsiveness may result from the purification procedure for LC. However, the finding that CGRP is a potent inducer of cAMP in LC is in accordance with the observation that LC are associated anatomically with epidermal nerves (2). We also measured the level of cAMP in thioglycolate-elicited Mϕ because suppression of murine Mϕ functions by CGRP has been reported, including that of antigen presentation (20), and cAMP induction by CGRP has been observed in rat peritoneal Mϕ (21) and a transformed murine Mϕ line (22). We found only a small induction of cAMP in murine peritoneal Mϕ, less than that seen in LC under the same conditions.

VIP was as potent as CGRP in inducing cAMP in LC. In our KC preparation cAMP was not highly increased by VIP. This finding differs from reports (4, 23) showing VIP-induced cAMP elevation in KC. This discordance may result from differences in the KC preparation protocol; these previous investigations used cultured KC, whereas we used fresh KC for analysis. SP failed to induce cAMP in either LC or KC, in agreement with the data that SP, in contrast to CGRP and VIP, stimulates phosphatidylinositol turnover, leading to PKC activation (24, 25).

We then examined whether the functional effects of CGRP on LC were also receptor-mediated, and, if so, whether cAMP was directly linked to that effect. The effect of CGRP on EC presentation of TAA for elicitation of DTH was blocked by

concomitant incubation in CGRP-(8–37), as was the effect of CGRP on the capability of EC to present PCC to the responsive clone A.E7, indicating that the effect is receptor-mediated. The failure of FK to significantly inhibit LC antigen presentation suggests that an increase in cAMP alone is not sufficient to inhibit LC antigen presentation. Because the effects of FK may not be specific (4), FK may, hypothetically, have other effects that counter the functional consequences of cAMP induction. However, the induction of B7-2 expression by peritoneal Mϕ and XS52 cells was suppressed by CGRP. This suppression of B7-2 expression may be one of the mechanisms responsible for the inhibitory effect of CGRP on antigen presentation. Interestingly, FK did not inhibit up-regulation of B7-2. Preliminary experiments examining the effect of CGRP on up-regulation of B7-1 expression by peritoneal Mϕ and XS52 cells suggest that CGRP may have little or no effect on B7-1 expression.

The adenylate cyclase–cAMP–protein kinase A pathway is generally considered to inhibit or down-regulate the amplification and effector phases of the immune response in T cells, B cells, and Mϕ (26). cAMP inhibits T-cell proliferation and IL-2 production, although some contradictory data have also been presented (27). cAMP and positive effectors of the adenylate cyclase system inhibit Mϕ functions, including lysosomal enzyme release, migration, phagocytosis, and cytotoxicity (28). However, FK, in contrast to CGRP, did not inhibit LC antigen presentation.

A role for cAMP as a signal inhibiting antigen presentation has been reported. Prostaglandin E_2 inhibits the expression of Ia molecules by peritoneal Mϕ cultured in lymphokine-containing conditioned medium, and this inhibition is mimicked by the addition of dibutyryl cAMP (29). Inhibition of IL-1 generation by prostaglandin E_2 may be via a cAMP-dependent pathway (26). Similarly, norepinephrine inhibits γ interferon-induced Ia expression by astrocytes, and this effect is mimicked by induction of intracellular cAMP by either dibutyryl cAMP or dipyridamole (30). Up-regulation of Ia expression on LC by either IL-2 or IL-6 is abrogated by cAMP analogs (31). In contrast, evidence exists that increased cAMP may be associated with increased potency of antigen presentation in some systems, including human monocytes (32, 33) and B cells (34, 35).

The CGRP receptor may be directly or indirectly coupled to multiple signaling systems, and in considering signal-transduction mechanisms, so-called "cross-talk" between cellular signaling pathways must be entertained (36). Examples of this exist in rat peritoneal Mϕ (21) and chicken skeletal muscle cells (9), and PKC may regulate adenylate cyclase activity (21, 26). The PKC activator phorbol ester inhibited CGRP-dependent cAMP formation in human ocular ciliary epithelial cells (37). Vasodilatation by CGRP has been shown to involve cAMP, cGMP, nitric oxide (NO), and ion channels (38, 39), and those factors influence one another.

In conclusion, we have shown that the intracellular level of cAMP is specifically increased after incubation of LC with CGRP. The effect of CGRP on inhibition of antigen-presenting capability is receptor-mediated and may involve, at least in part, inhibition of B7-2 expression. These effects cannot be reproduced by nonspecific elevation of cAMP in LC and may involve alternative signaling pathways. These results support the hypothesis that the functional repertoire of LC is regulated by CGRP.

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