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Novel Cyclic Peptides Containing a γ-Glutamyl Residue Induce IL-1 Secretion from Human Peripheral Blood Mononuclear Cells

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Abstract: Three synthetic cyclic tripeptides, each containing a γ-glutamyl residue were shown to induce the production of interleukin-1 (IL-1) from human peripheral blood mononuclear cells (PBMC). In addition, the cyclic peptide cyclo[Glu(Lys-β–Ala-)-OH] (cG-2) was capable of eliciting IL-1 production from PBMC dose-dependently.

In a previous paper[1] we reported the synthesis of two cyclic peptide homologs of glutathione (GSH): cyclo[Glu(Cys-β–Ala-)-OH] (cG-1), and cyclo[Glu(Cys-Gaba-)-OH] (cG-3). Recently we synthesized another cyclic peptide: cyclo[Glu(Lys-β-Ala-)-OH] (cG-2) in which the Cys residue of cG-1 was replaced by a lysyl residue. The intent was to investigate the difference in biological activity caused by the substitution of the Cys residue with an amino acid residue bearing a basic side chain.

It has been known that GSH has immunological activities of enhancing mitogen- or cytokine-stimulated lymphoproliferation and eliciting antigen-induced inflammation.[2-7] In addition, LPS-stimulated macrophages/monocytes produce mainly IL-1β, an important cytokine.[8] It is known that IL-1 has important roles in immunomodulation such as stimulating T and B lymphocytes,[9,10] radioprotecting effects,[11] and reducing the mortality from bacterial and fungal infections in mice.[12] IL-1 also induces fever, sleep, anoresia, and hypotension, and has been implicated as a mediator of disease.[13] In clinical trials, IL-1 increases the number of bone marrow precursor cells, platelets, and neutrophils.[14, 15] Thus, we were interested in testing these cyclic peptides containing a γ-glutamyl residue in the induction of IL-1 secretion from PBMC and murine peritoneal adherent cells.

The rationale for us to prefer synthesizing cyclic peptides to linear peptides was described.[16,17] The scheme for the synthesis of the cyclic peptide cG-2 is outlined in Figure 1. The non-chiral β-Ala was chosen as the *C*-terminal residue in order to avoid possible racemization during the cyclization step. The linear peptide was synthesized in solution by stepwise coupling of the protected amino acid residues using the DCC/HOBt method.[18] Selective removal of the *C*-terminal phenacyl group by Zn/acetic acid treatment afforded the free acid **4** which was esterified with pentafluorophenol/DCC to give the active ester **5**. After removing the Boc group of

the active ester the TFA salt was subjected to cyclization at elevated temperature in high dilution [19] to afford high yield (84%) of the protected cyclic peptide **6**.[20] After the removal of the Z and Bzl groups by catalytic hydrogenation, the crude target peptide was purified to homogeneity by HPLC. The monomeric structures of both the protected and deprotected cyclic peptides were confirmed by fast atom bombardment mass spectrometry.[20]

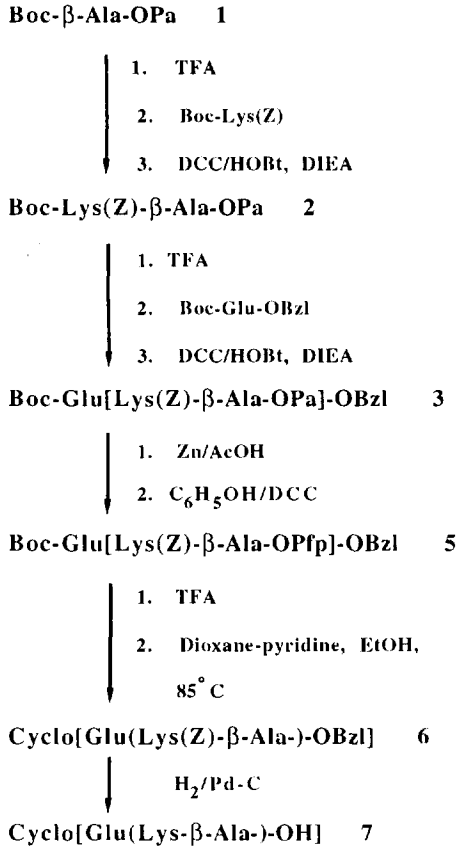

Figure 1

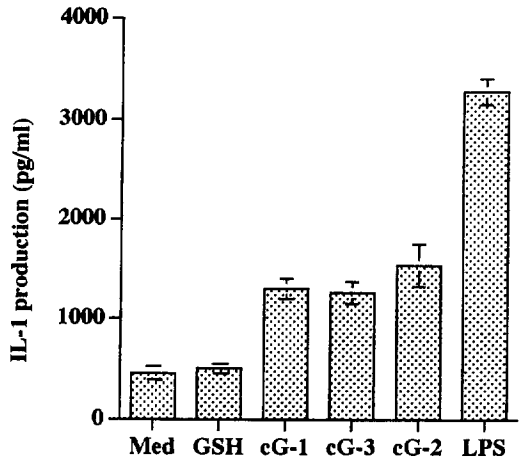

Figure 2. Induction of IL-1 secretion by cyclic peptides. Human PBMC (2 x 10^6/ml) were stimulated with cyclic peptides (1 μg/ml) or LPS (0.1 μg/ml) in 10% FCS-RPMI-1640 medium for 24 h at 37°C. The supernatants were collected and assayed for IL-1 by the ELISA method. Med, culture medium only. Data represent mean ± SE of four replicates; $p < 0.01$.

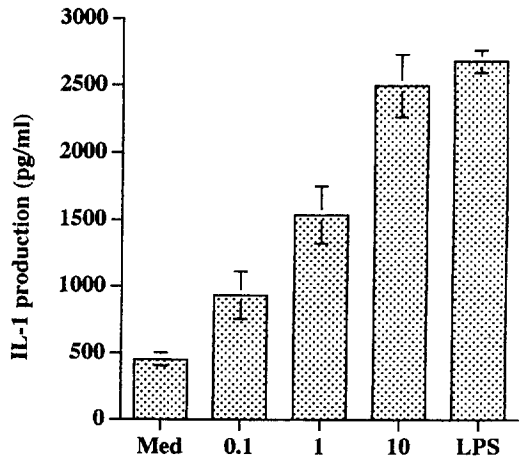

Figure 3. Dose-dependent response of cG-2 on IL-1 production human PBMC. The cells (2 x 10^6/ml) in 10% FCS-RPMI 1640 medium were stimulated with 0.1, 1, 10 μg/ml of cG-2 or with 0.1 μg/ml of LPS for 24 h at 37°C. Data represent mean ± SE of four experiments; $p < 0.01$.

The cyclic peptides were examined for their abilities to induce IL-1 production which is an important factor for cell proliferation. Human PBMC were stimulated either with LPS or cyclic peptides and cultured for 24 hours. The supernatants collected from cyclic peptides cG-1, cG-2, and cG-3 treated groups produced significantly higher IL-1 levels than that of the medium control by the ELISA assay (Figure 2).[21] At the concentration of 1 μg/ml, the IL-1 level induced by cG-2 was greater than that of cG-1 and cG-3, but much lower than that of LPS. In addition, the IL-1 production by cyclic peptides was increased dose-dependently by ELISA and bioassay (data not shown). At a higher concentration (10 μg/ml), the IL-1 level induced by cG-2 (2498 ± 235 pg/ml) was similar to that of LPS at a concentration of 1 μg/ml (2680 ± 85 pg/ml) (Figure 3). The supernatants collected from cG-1 treated murine peritoneal adherent cells were shown to have significantly higher IL-1 activities than that of medium control (data not shown).

In present results, glutathione did not induce IL-1 activity in monocytes (Figure 2). Since GSH is widely distributed in animal tissues, plants, and microorganisms and is present in high concentrations (0.1-10 mM), it is the most prevalent cellular thiol.[22] As an important component in maintaining the function of cell, soluble GSH unlikely has any stimulatory effect on IL-1 production.

In this work, all of the three cyclic peptides were capable of enhancing cell proliferation (data not shown) and induced secretion of IL-1 by human PBMC or murine macrophages and the response of cG-2 was dose-dependent. The IL-1 production induced by cG-2 was greater than that by cG-1 and cG-3, indicating that the structure-activity relationship of these three cyclic peptides was involved. The cyclic peptides induced a higher IL-1β production than TNF-α production by PBMC (data not shown). cG-1 (100 μg/ml) induced IL-1β productions (2800 pg/ml) and TNF-α productions (450 pg/ml) from one experiment. The treatment of PBMC with cyclic peptides, cG-1, cG-2 and cG-3 for 24 h did not increase cell death as compared with their medium control by trypan blue exclusion. In addition, LAL gelation test showed that all three cyclic peptides up to the concentration of 100 μg/ml were negative for endotoxin.[21]

It is noteworthy that IL-1 production can be induced by the treatment of monocytes with cisplatin[23] or a synthetic cyclic octapeptide cyclo[Tyr-Ser-Gln-Val-Leu-Phe-Lys-Gly-] (cTNF-2).[17] However, the structure-activity relationships which influence the immunological activities of these compounds are not clear. Both the cyclic peptides cTNF-2 and cG-2 possess a lysyl residue, and are capable of inducing IL-1 secretion from PBMC dose-dependently. Thus, we envisage that the lysyl residue, which bears a positive charge at the ε-amino group at physiological pH, may be involved in enhancing the induction of IL-1 secretion. However, further synthesis and testing of cyclic peptides containing a lysyl residue must be done to support this hypothesis. These cyclic peptides may be useful as potential immunomodulators under further studies.

Acknowledgments

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20. *Cyclo[Glu-(Lys(Z)-β–Ala)-OBzl]* **6**. m.p. 230-231°C; MS: (FAB, Xe beam) (m/z, %RA): 551.10 $[M-H]^-$; Anal. calc. for $C_{29}H_{36}N_4O_7 \cdot 0.5\ H_2O$: C 62.02, H 6.64, N 9.98. Found: C 62.08, H 6.65, N 9.86.
Cyclo[Glu-(Lys-β–Ala)-OH] **7**. The protected cyclic peptide **6** (0.30 g) was dissolved in MeOH/H_2O/AcOH and hydrogenated over Pd-C (0.20 g) for 16 h, filtered over Celite, and evaporated. The residue was purified by semi-preparative HPLC (Vydac TP 201, 1.0 x 25 cm) using H_20 as eluent to afford a white solid (0.10g, 57%);

mp 274-275°C; MS: (FAB, Xe beam) (m/z, %RA): 327.17 $[M-H]^-$ Anal. calc. for $C_{14}H_{24}N_4O_5 \cdot 1.5\ H_2O$: C 47.31, H 7.68, N 15.76. Found: C 46.92, H 7.66, N 15.60.

21. The IL-1 activity was determined by thymocyte proliferation assay. Thymocytes from C3H/HeJ mice obtained from Jackson Laboratory (Bar Harbor, ME) were suspended in 10%FCS-RPMI-1640 culture medium and stimulated with PHA and supernatants in the wells of microtiter plates for 72 h incubation. ^{3}H-thymidine (0.5 μC_i) was added to each well at the end of 48 hr incubation. The IL-1 activity was measured by scintillation counting as cpm.[9] The IL-1β productions from human PBMC samples including medium control and cyclic peptides treatment were determined by an ELISA kit (Cistron, Pine Brook, NJ). Briefly, samples and IL-1β standards were assayed in the wells of microtiterplate precoated with monoclonal anti-IL-1β antibody. After incubation for 20 min at 37°C, the unbound components from the samples were removed by washing with PBS/tween 20 solution. Following the binding of polyclonal rabbit antibody to bound IL-1 after another incubation, a third antibody goat anti-rabbit IgG conjugated with horseradish peroxidase was added to the wells and incubated again at room temperature. The color product developed after the addition of substrate was measured with a spectrophotometer at 450 nm. The IL-1 levels were then determined from a standard curve. The TNF-α production from human PBMC samples was determined by an ELISA kit (R&D, Minneapolis, Minnesota) similar to IL-1 ELISA assay.

Limulus amebocyte lysate test: To detect the possible contamination of endotoxin in the cyclic peptides preparation, limulus amebocyte lysate (LAL) test (Biowhittaker Inc., Walkersville, MD, USA) was used. Cell culture medium containing cG-1, cG-2 and cG-3 (0.1-100μg/ml each) and cell culture supernatants were test for gelation after incubating with LAL reagent for 1hr at 37°C. The results were analyzed and compared with positive controls.

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