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Immunomodulatory effects of peptide T on Th 1/Th 2 cytokines

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Abstract

Peptide T is an octapeptide from the V2 region of HIV-1 gp120. It has been shown to resolve psoriatic lesions — an inflammatory skin disease. The mechanisms of anti-inflammatory actions of peptide T are not well understood. Th1 cytokines such as IL-2, and IFN-γ are upregulated in psoriasis. These cytokines play a key role in the inflammatory and proliferative processes of psoriasis. The effects of peptide T on Th1 and Th2 cytokines were studied in order to elucidate the mechanisms of antiinflammatory actions of peptide T. It was observed that peptide T at 10^{-8} M induces IL-10 production by the human Th2 cell line and PBMC ($P < 0.05$, ANOVA). Also peptide T at 10^{-9} M concentration significantly inhibited IFN-γ production by PBMC ($P < 0.001$, ANOVA). Anti IL-10 antibody inhibited the anti-IFN-γ effect of peptide T ($P < 0.05$, *t*-test). Our study shows that peptide T induces IL-10 production and inhibits IFN-γ production. IL-10 is a potent anti-inflammatory cytokine. It inhibits IL-2 and IFN- γ production from the T cells and downregulates the expression of TNF-α in the antigen presenting cells. Recently, IL-10 has been shown to resolve psoriatic lesions. The effects of peptide T on IL-10 and IFN-γ production provides a plausible explanation for its clinical efficacy in psoriasis.

1. Introduction

Peptide T is a ligand for the CD4 receptor [19]. It is believed that peptide T is a competitive

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antagonist of gp120 and prevents binding of HIV to the CD4 receptor [19]. Recently it has been shown to act at CCR5 chemokine receptors [21]. The first report regarding the therapeutic efficacy of peptide T was an incidental observation that psoriasis cleared during peptide T treatment in an AIDS patient [29]. Subsequently several reports have reproduced the efficacy of peptide T in psoriasis and psoriatic arthritis [5,12].

How peptide T counters the inflammatory process of psoriasis is largely unknown. It has been suggested that peptide T might block the CD4 receptor, thus preventing the penetration of putative psoriasis-causing retrovirus. Peptide T might also affect the function of CD4+ cells found in the lesional skin of psoriasis [9]. Other mechanisms might also be plausible like competitive inhibition of VIP and interactions with somatostatin [5,9,10,26].

Activated T cells, along with their cytokines, play a key role in the pathogenesis of psoriasis [2,28]. Over-expression of proinflammatory cytokines is a documented feature of psoriasis. Upregulation of Th1 cytokines such as IL-2 and IFN-γ in psoriatic lesions both at cellular and molecular levels had been reported by several authors [13,27]. These observations are supported by the beneficial effects of immunosuppressive drugs which downregulate Th1 cytokines such as Cyclosporin A [25] and FK506 [8].

An alternative approach to counter an inflammatory process induced by Th1 cytokines is to promote a Th2 anti-inflammatory cytokine such as IL-10. IL-10 is produced by Th2 cells, and inhibits IFN-γ production from the Th1 cells [7,23]. Recently therapeutic efficacy of IL-10 in psoriasis has been reported [1]. Peptide T has also been shown in other inflammatory diseases, e.g., AIDS to increase IL-10 levels and decrease IFN-γ levels [20].

In this study we have evaluated the effects of peptide T on IL-10 and IFN-γ production to elucidate the antiinflammatory properties of peptide T.

2. Material and methods

2.1. Peptide T

Peptide T (d-[A]STTTNYT-NH2) was obtained from Phoenix laboratory, Mountain View, CA. It was >95% pure.

2.2. Study population

Ten psoriatic patients (age: median 39, range 22–59 years, sex: 3 female, 7 male) and 10 normal individuals (age: median 38, range 24–58 years, sex: 3 female, 7 male) were included in this study. Patients had moderate to severe psoriasis without any treatment for at least three months before blood donation.

2.3. Effect of peptide T on Th2 cytokine production

Peripheral blood mononuclear cells (PBMC) were obtained from 10 psoriatic patients and 10 normal healthy individuals as a control group. Patients and controls were age (29–49 years) and sex matched. This study was approved by Institutional Review Board and each individual

signed an informed consent form. One million PBMC from each individual were stimulated with ConA (3 μg/ml) along with various concentrations of peptide T (10^{-6}–10^{-12} M) for 48 h at 37°C. Supernatants were collected and frozen at −70°C. Human IL-10 ELISA kits from Endogen, MA, were used for IL-10 detection in the supernatant.

A human Th2 cell line was also used. This cell line was a gift from Dr T. Schall, DNAX Corp., Palo Alto, CA. The cell line was maintained in our laboratory by adding feeder layer and stimulants at 14 day intervals. The feeder layer (A) consisted of irradiated human peripheral blood lymphocytes (10^6/ml), an irradiated Epstein–Barr virus transformed B cell line (10^5/ml) and PHA (0.1μg/ml) in YM® medium and T cell clone (B) used at 2×10^5/ml. Both (A) and (B) were mixed together and incubated at 37°C in a humidified 5% CO_2 incubator. After 3–4 days, IL-2 was added at a concentration 20 ng/ml. Once the cells were in synchronous growth, peptide T at 10^{-6}–10^{-12} M concentrations was added and incubated at 37°C for 48 h. Supernatants were collected and frozen at −70°C. Human IL-10 ELISA kits from Endogen, MA, were used for IL-10 detection in the supernatant.

2.4. Effect of peptide T on Th 1 cytokine production

Peripheral blood mononuclear cells were obtained from 10 psoriatic patients and 10 normal healthy individuals as a control group. One million PBMC of each individual were stimulated with PHA (5 μg/ml) along with various concentrations of peptide T (10^{-6}–10^{-12} M) for 48 h at 37°C. Supernatants were collected and frozen at −70°C. Anti-IL-10 antibody (20–50 ng/ml) (R&D Systems, MN) was used to assess its effect on the anti-IFN-γ effect of peptide T. Human IFN-γ ELISA kits from Endogen, MA, were used for IFN-γ detection in the supernatant.

3. Results

Fig. 1 shows that peptide T significantly increases IL-10 production by PBMC both in

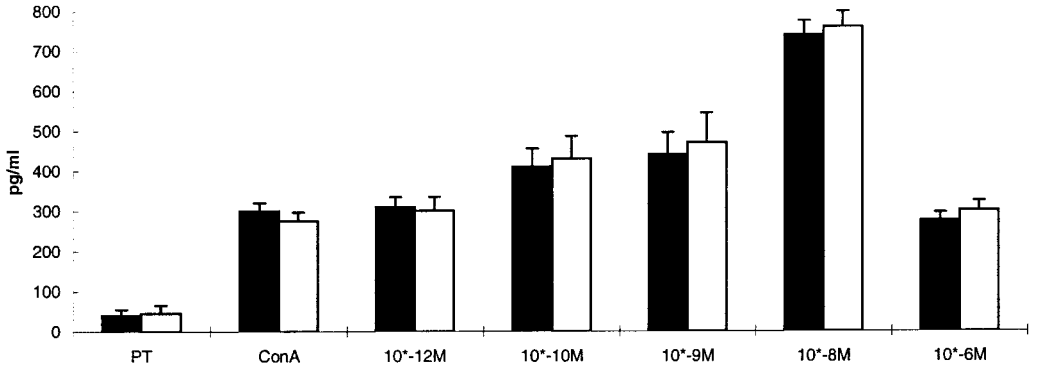

Fig. 1. Effects of peptide T on IL-10 production by PBMC of 10 normal healthy individuals and 10 psoriatic patients. PT: peptide T at 10^{-8} M alone; ConA: ConA at 3μg/ml; PT12–PT6: Peptide T^{-12} M–peptide T^{-6} M concentration. ■, psoriatic patients; □, normal individuals. $P < 0.05$ at peptide T 10^{-8}M concentration.

psoriasis patients and the control group. The effect is significant at 10^{-8} M ($P < 0.05$, ANOVA). Each assay was done in duplicate wells. Supernatants from each well were collected and each supernatant was tested in duplicate by ELISA assay. Therefore, each experimental set up was tested in quadruplicate by ELISA for reproducibility assessment. Variation was within 5%. Fig. 2 shows that peptide T at 10^{-8} M concentration significantly increases IL-10 production by the Th2 cell line ($P < 0.05$, ANOVA).

Fig. 3 shows that peptide T at 10^{-9} M concentration significantly inhibits IFN-γ production in both psoriatic patients and the control group ($P < 0.001$, ANOVA). Reproducibility of the IL-10 production by Th2 cell line and IFN- γ production by PBMC were assessed as described for IL-10 production by human PBMC. Variation was 4%. There was no difference in IL-10 and IFN-γ production capability by peptide T among psoriatic and healthy individuals. Fig. 4 shows that anti-IL-10 antibody inhibited the anti-IFN-γ effect of peptide T. This suggests that the effect of peptide T on the production of these cytokines is an inherent property of this drug, and in the lymphocytes of psoriatics these functions remain unchanged.

4. Discussion

Our results shows that peptide T upregulates production of IL-10 by human PBMC as well as by the human Th 2 cell lines. Peptide T downregulates IFN-γ production by PBMC. The anti-IFN-γ properties of peptide T are significantly reduced by anti-IL-10 antibodies. Reduced levels of IFN-γ in the supernatants of the PBMC treated with peptide T is concurrent with the higher levels of IL-10. IL-10 is known to inhibit IFN-γ production [7,23].

It is becoming increasingly clear that the clinical course of autoimmune diseases are influenced by the balance of Th1 and Th2 lymphocyte subsets which are generated during the response. Activated T cells along with their proinflammatory cytokines such as IL-2, IFN-γ and TNF-α play key roles in the inflammatory and proliferative process of psoriasis [2,13,27,28]. IFN-γ is mitogenic to keratinocytes [16] and inhibits IL-10 expression [24]. Patients treated with systemic IFN and IFN elevating drugs like lithium are known to induce or exacerbate psoriasis [14,30]. Subcutaneous perfusion of TNF-α causes proliferation of keratinocytes and endothelial cells [15], which are salient histological features of psoriasis. In addition both IFN-γ and TNF-α induce potent chemokines (IL-8, RANTES, fractalkine) and

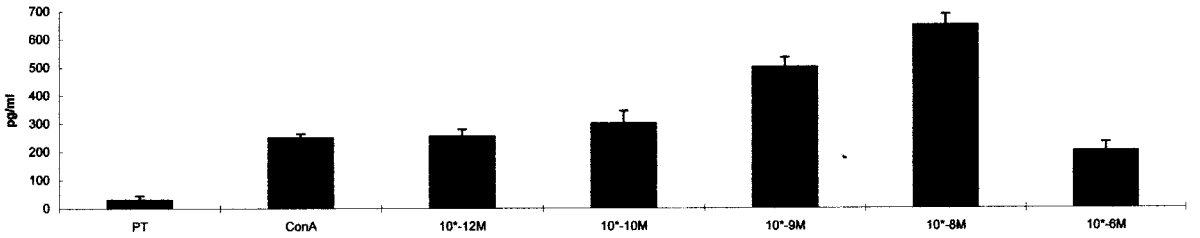

Fig. 2. Effects of peptide T on IL-10 production by a human Th2 cell line. PT: peptide T at 10^{-9} M alone; ConA: ConA at 3μg/ml, 10^{*-12} M–10^{*-6} M: ConA at 3μg/ml + peptide T at 10^{-12} M–peptide T at 10^{-6}M concentration. $P < 0.05$ at peptide T 10^{-8}M concentration.

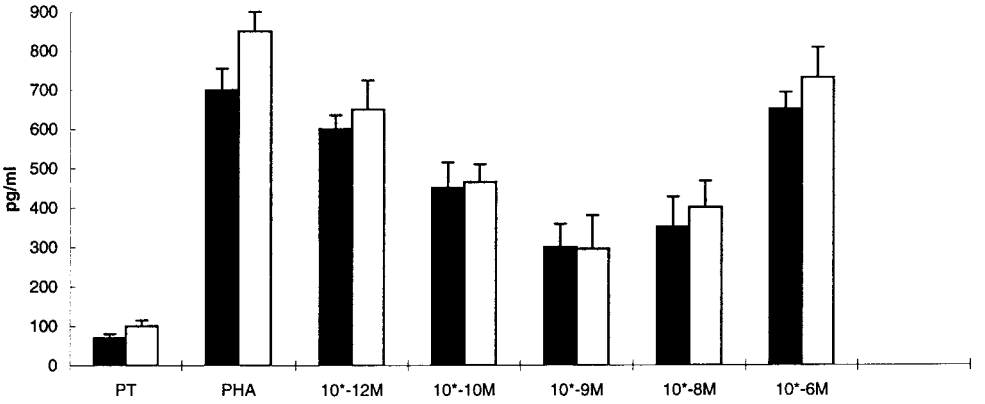

Fig. 3. Effects of peptide T on IFN-γ production by PBMC of 10 normal healthy individuals and 10 psoriatic patients. PT: peptide T at 10^{-9} M alone; PHA–PHA at 5 μg/ml; 10^{*-12} M–10^{*-6}M: PHA at 5 μg/ml + peptide T at 10^{-12} M–peptide T at 10^{-6} M concentration. ■, psoriatic patients, □, normal individuals. $P < 0.001$ at peptide T 10^{-9}M concentration.

adhesion molecules [11,17,18,22]. Thus they play a key role in recruiting and retaining the inflammatory infiltrates. Peptide T inhibits the monocyte and lymphocyte chemotactic properties of RANTES [17]. This is consistent with the observation that peptide T acts at CCR5 chemokine receptor [21]. IL-10 is one of the several cytokines which influence the differentiation of T-helper cell subsets and represents a target for therapeutic intervention. IL-10 has a great impact on immunoregulation. It promotes the development of a Th2 type cytokine pattern by inhibiting the IFN-γ production of T lymphocytes and natural killer cells particularly via the suppression of IL-12 synthesis in accessory cells [3]. In addition, IL-10 affects antigen presenting cell functions. It inhibits the antigen-presenting capacity of monocytes/macrophages, dendritic cells and suppresses proinflammatory cytokine production [4,6]. In a recent report therapeutic efficacy of IL-10 in psoriasis has been demonstrated [1]. Subcutaneous administration of IL-10 resulted in significant clinical and histological improvement. IL-10 therapy also suppressed production of TNF-α and expression of HLA-DR

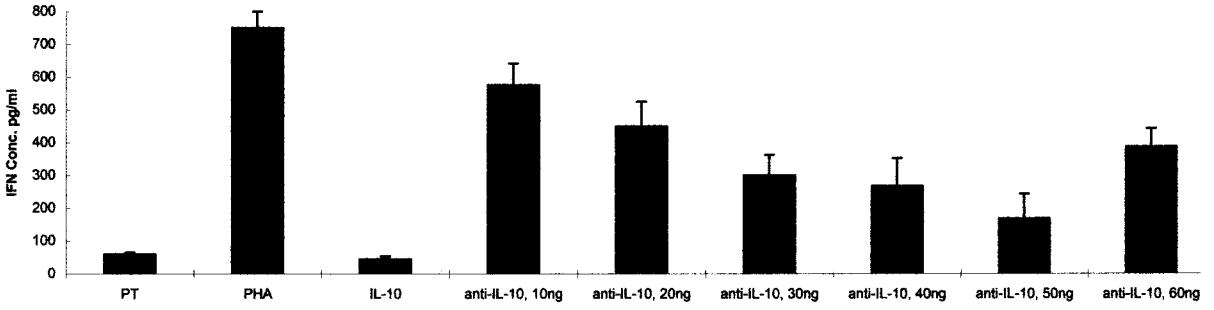

Fig. 4. Effects of anti-IL10 antibodies on anti-IFN-γ effect of peptide T. PT: peptide T at 10^{-9} M alone; PHA; PHA at 5μg/ml; IL-10: IL-10 alone at 50 ng/ml; anti-IL-10, 10 ng–60 ng: PHA (5 μg/ml), anti-IL-10 antibody at 10–60 ng/ml and peptide T at 10^{-9} concentration.

in the monocytes [1]. Peptide T has also been shown to increase IL-10 levels and suppress IFN-γ levels [20].

Our study shows that peptide T upregulates IL-10 production; this provides a plausible explanation for its efficacy in inflammatory conditions like psoriasis and psoriatic arthritis. Peptide T is unique with respect to its Th2 cytokine promoting function. It is likely that peptide T might be beneficial for other Th1 cytokine mediated inflammatory diseases such as rheumatoid arthritis, ulcerative colitis, etc.

Acknowledgements

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