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The expression of mCTLA-4 in skin lesion inversely correlates with the severity of psoriasis

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ABSTRACT

Background: Psoriasis is a chronic inflammatory disease characterized by epidermal hyperplasia and increased T cell infiltration. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is a key factor that affects T cell function and immune response. However, whether the expression of CTLA-4 affects the severity of psoriasis is still unknown.

Objective: The aim of the project was to investigate the correlation between the expression of CTLA-4 and the severity of psoriasis.

Methods: The plasma soluble CTLA-4 levels and membrane CTLA-4 expression were measured by enzyme-linked immunosorbent assay and immunohistochemistry analysis in mild, moderate and severe psoriasis patients, respectively. Imiquimod-induced mouse model of psoriasis was treated with CTLA-4 immunoglobulin fusion protein (CTLA-4 Ig) or anti-CTLA-4 antibody. Epidermal thickness and infiltrating CD3+ T cell counts were evaluated.

Results: The plasma soluble CTLA-4 levels had no significant difference among mild, moderate, and severe patients ($p > 0.05$). However, the membrane CTLA-4 expression in skin was significantly higher in mild psoriasis patients compared to moderate and severe psoriasis patients (17652.86 ± 18095.66 *vs* 6901.36 ± 4400.77 *vs* 3970.24 ± 5509.15, $p < 0.001$). Furthermore, in imiquimod-induced mouse model of psoriasis, the results showed that mimicking CTLA-4 function improved the skin phenotype and reduced epidermal thickness (172.87 ± 28.25 *vs* 245.87 ± 36.61 μm, n = 6, $p < 0.01$) as well as infiltrating CD3+ T cell counts (5.09 ± 3.45 *vs* 13.45 ± 4.70, $p < 0.01$) compared to control group. However, blocking CTLA-4 function aggregated the skin phenotype including enhanced epidermal thickness and infiltrating CD3+ T cell counts compared to control group.

Conclusion: These results indicated that the expression of mCTLA-4 in skin lesion inversely correlated with the severity of psoriasis and CTLA-4 might play a critical role in the disease severity of psoriasis.

1. Introduction

Psoriasis is an immune-mediated chronic inflammatory disease characterized by abnormal epidermal differentiation, hyperproliferation, angiogenesis, and increased T-cell infiltrates. As a chronic relapsing and remitting inflammatory skin disease, it affects approximately 1–3% of the population [1].

T cell activation requires two discrete signals: one signal delivered by a T cell receptor and the other one occurred when co-stimulatory receptors interact with their ligands. CD28, a major co-

Abbreviations: CTLA4, Cytotoxic T lymphocyte antigen-4; sCTLA-4, soluble Cytotoxic T lymphocyte antigen-4; mCTLA-4, membrane Cytotoxic T lymphocyte antigen-4; IOD, Integrated option density; IMQ, Imiquimod; PASI, psoriasis area and severity index; Treg, regulatory T.

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stimulatory receptor, delivers a potent co-stimulatory signal to T cells, whereas Cytotoxic T lymphocyte antigen-4 (CTLA-4), a homologue receptor of CD28, is a key inhibitory receptor that affects T cell function and plays a critical role in the priming phase of the immune response by delivering negative signals to T cells [2,3]. It was reported that germline haploinsufficiency of CTLA-4 caused lymphoproliferation and autoimmunity in humans [4,5]. There are two protein isoforms that have been described in humans: a soluble monomeric form (sCTLA-4) and the canonical transmembrane homodimer (mCTLA-4). Both forms come from alternative mRNA splicing of the nascent CTLA-4 transcript and bind to the immunoglobulin superfamily ligands CD80/CD86 [6].

The sCTLA-4 lacks the transmembrane domain of the full-length CTLA-4 receptor and carries a different C-terminal amino acid sequence, but crucially retains the capacity to engage with ligands CD80/CD86 [7]. Previous studies have shown that sCTLA-4 from with autoimmune diseases regulated T-cell responses [8]. Therefore, the expression of sCTLA-4 may have an impact on disease severity.

As for mCTLA-4, in resting T cells, it is found in the intracellular compartment. Following T cell activation through CD28 binding, mCTLA-4 is expressed on the surface of T cells [9,10]. The stronger the stimulation, the more mCTLA-4 is expressed and translocated to the T cell surface [11]. Besides, on cell membrane, mCTLA-4 binds to CD80/CD86 with a higher affinity than the co- stimulatory receptor CD28, intercepting the binding of the latter and providing an inhibitory signal to T cells. Suárez-Fariñas M et al. have shown that CTLA-4 was expressed on keratinocytes and some dermal cells in lesion skin while little or none expression on non-lesion and normal skin [12]. However, it is still unknown whether mCTLA-4 plays any role in the severity of psoriasis.

In this study, the expression of sCTLA-4 and mCTLA-4 was detected in the mild, moderate and severe psoriasis. CTLA-4 function was activated or blocked by specific agents *in vivo* to evaluate skin phenotype and histological changes in the mouse model of psoriasis.

2. Materials and methods

2.1. Subjects

All patients in this study were diagnosed of plaque psoriasis by a dermatologist based upon clinical presentation and histologic examination. Patients who were treated with oral corticosteroids, systemic retinoid, immunosuppressants (such as methotrexate and cyclosporine), biologics, photo-/photochemotherapy or topical treatment were excluded from this study. All patients in our study were small plaque (Asian) psoriasis (plaques are usually <2 cm in diameter) [13]. Patients were grouped by PASI (psoriasis area and severity index) score [14] into mild (PASI < 10), moderate ($10 \leq$ PASI < 20), and severe (PASI ≥ 20), respectively. Plasma and skin biopsy tissues were obtained from patients before initiating any treatment. Demographic features from all patients including age, sex, BMI, age of first onset, duration, comorbidities, family, smoking history were recorded. Totally, 60 plasma samples and 79 lesion biopsy tissues from plaque psoriasis patients and 9 normal skin biopsy tissues from healthy subjects were studied. This study was approved by the ethics committees of Xiangya hospital of Central South University, Changsha, Hunan, China, and informed consent was obtained from all subjects.

2.2. Plasma soluble CTLA-4 measurement

Plasma (EDTA) was stored at $-20\,^{\circ}$C until use. Plasma sCTLA-4 levels were measured by enzyme-linked immunosorbent assay (ELISA) using human soluble CTLA-4 (sCLTA-4) kit (E-bioscience, Vienna, Austria), following manufacturer's instructions. 10 fold-diluted samples were blindly tested in duplicates to generate mean values used in the analysis.

2.3. Histological evaluation and immunohistochemistry (IHC)

Skin samples were obtained with 3 mm surgical biopsy from representative lesions. Each specimen was embedded in paraffin and split for routine histopathology and immunohistochemistry analysis on paraffin slicing machine-cut 3 μm sections. Sections were stained with hematoxyline and eosin stain (H&E stain) for histological evaluation. Immunohistochemistry was performed according to previous study [15]. Briefly, sections were incubated with monoclonal antibody: CTLA-4 (sc-376016, santa cruz, Dallas, Texas, USA) or CD3 (ab-16669, abcam, Cambridge, Massachusetts, USA) at $4\,^{\circ}$C overnight. Bound antibodies were detected by using a conventional streptavidin-biotin method according to manufacturer's instructions (S-A/HRPkit, ZSGB-BIO, beijing, China). The reaction was visualized by DAB+ Chromogen, and slides were counterstained with hematoxylin.

2.4. Immunohistochemical analysis

Immunostained sections were characterized semi-quantitatively by digital image analysis using the Image Pro-Plus (Image Pro-Plus 6.0 image-analysis software) by using the method as previously reported [15,16]. Briefly, images at 1360×1024 pixel resolution at $400 \times$ magnification were obtained with an Olympus CX41 microscope fitted with a micro Image video camera (Mshot). A series of 7 random images on several sections were taken for each immunostained parameter to obtain a mean value for statistical comparison. Staining was defined via color intensity, and a color mask was made. The mask was then applied equally to all images, and measurements were obtained [15,16]. The measurement parameter included integrated option density (IOD) and immunostaining positive cell counts. The optical density was calibrated and the area of interest was set through: hue $9 \sim 25$, saturation $0 \sim 255$, intensity $0 \sim 168$, and then the values were counted. Two independent examiners evaluated these sections without prior knowledge of the clinical status. CTLA-4+ IOD, CTLA-4+ T Cells, CD3+ T Cells, CTLA-4+ IOD divided by CTLA-4+ T Cells and CTLA-4+ IOD divided by CD3+ T Cells were calculated.

2.5. Mouse model of psoriasis-like skin inflammation

8-week-old BALB/c female mice (purchased from the department of laboratory animals of Central South University) were used. Imiquimod (IMQ) was used to induce skin inflammation as previously described [17]. A daily dose of 62.5 mg of 5% IMQ cream (Med-shine Pharmaceutical Co., Ltd., Sichuan, China) was applied to the shaved back of mice, translating in a daily dose of 3.125 mg of the active compound. Vaseline was used as control. All experiments were performed according to the Animal Care and Use Committee guidelines of Xiangya medicine school of Central South University.

2.6. CTLA-4 Ig and anti-CTLA-4 antibody treatment

Mice were treated with vaseline, IMQ and the CTLA-4 Ig (IMQ+ CTLA-4 Ig), IMQ alone, or IMQ and the anti-CTLA-4 antibody (IMQ+ anti-CTLA-4 mAb) for 7 days. For each group, $n = 6$. For the treatment, two groups of mice were administered with CTLA-4 Ig (BP0099, Bio X cell, West Lebanon, New Hampshire, USA) or anti-CTLA-4 antibody (anti-CTLA-4 mAb) (BP0164, Bio X cell, West Lebanon, New Hampshire, USA) at a dose of 1000 μg day 0, 3, 6 via intra-peritoneal injection, according to previous studies [18,19]. On

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