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ORIGINAL ARTICLE/ARTICLE ORIGINAL

# Altered immune responses in patients with chronic mucocutaneous candidiasis

## *Altération des réponses immunitaires chez les patients atteints de candidose chronique cutanéo-muqueuse*

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**KEYWORDS**
Chronic mucocutaneous candidiasis;
Cellular immunity;
Lymphocyte transformation;
Cytokines

**Summary**
*Objective.* — The purpose of this study was to investigate the lymphocyte transformation responses and cytokine secretion of peripheral blood mononuclear cells (PBMC) from patients with chronic mucocutaneous candidiasis (CMC).
*Methods.* — Phytohaemagglutinin (PHA) mitogen and *Candida albicans* (*C. albicans*) antigen proliferation assays were performed by culturing PBMCs in RPMI 1640. The levels of interleukin (IL)-2, IL-10, IL-17 and interferon (IFN)-γ present in the supernatant of cultures were determined using enzyme-linked immunosorbent assay (ELISA).
*Results.* — The results showed that most patients (92.3%) had a low proliferative response to *C. albicans* antigens and PHA. PBMCs from CMC patients produced lower levels of T (h)-1 cytokines IL-2 ($78.5 \pm 59.8$ pg/mL) and IFN-γ ($115.1 \pm 43.3$ pg/mL) in response to *Candida* antigens when compared to controls (Il-2: $177 \pm 103.6$ pg/mL; IFN-γ: $330.3 \pm 21.6$ pg/mL) ($P < 0.05$). Conversely, we observed a partial enhancement of IL-10 in the patients ($213.7 \pm 86.1$ pg/mL). Production of IL-17 indicated no significant differences between patients and controls when stimulated by *Candida* antigens ($21.5 \pm 8.6$ pg/mL versus $32.4 \pm 12.2$ pg/mL) and PHA ($27.7 \pm 11.5$ pg/mL versus $36.2 \pm 9.1$ pg/mL), respectively.
*Conclusion.* — These findings suggest that *Candida* antigens trigger a Th2 instead of Th1 cytokine response in patients with CMC. For better understanding, further studies require on a larger number of patients into the future.


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**MOTS CLÉS**
Candidose chronique cutanéo-muqueuse ;
L'immunité cellulaire lymphocytaire ;
Transformation ;
Cytokines

**Résumé**
*Objectif*. — Le but de cette étude était d'étudier la transformation lymphocytaire et la sécrétion cytokiniques des cellules mononuclées du sang périphérique (PBMC) chez des patients atteints de candidose chronique cutanéo-muqueuse (CMC).
*Matériel et méthodes*. — Les tests de prolifération avec la phytohémagglutinine (PHA) et avec les antigènes de *Candida albicans* (*C. albicans*) ont été effectués par la culture de PBMCs en RPMI 1640. Les niveaux de l'interleukine (IL) -2, IL-10, l'IL-17 et de l'interféron (IFN) -γ présents dans le surnageant des cultures ont été déterminés à l'aide test immuno-enzymatiques (Elisa).
*Résultats*. — Les résultats ont montré que la plupart des patients (92,3 %) avaient une faible réponse proliférative de *C. albicans* antigènes et à la PHA. Les PBMCs des patients atteints de CMC produisent des niveaux inférieurs de T (h)-1 cytokines IL-2 (78,5 ± 59,8 pg/mL) et L'IFN-γ (USD 115,1 millions ± 43,3 pg/mL) en réponse aux *Candida* antigènes de *Candida* comparés aux contrôles (Il-2 : 177 ± 103,6 pg/mL ; IFN-γ : 330.3 ± 21,6 pg/mL) ($p < 0,05$). Inversement, nous avons observé une amélioration partielle de l'IL-10 chez les patients (213,7 ± 86,1 pg/mL). La production de l'IL-17 ne montrait aucune différence significative entre les patients et les contrôles lorsque stimulé par *les* antigènes de *Candida* (21,5 ± 8,6 pg/mL versus 32,4 ± 12,2 pg/mL) et la PHA (27,7 ± 11,5 pg/mL versus 36,2 ± 9,1 pg/mL), respectivement.
*Conclusion*. — Ces résultats suggèrent que les antigènes de Candida déclenchent une production cytokinique Th2 au lieu de Th1 chez des patients avec CMC. Pour une meilleure compréhension, d'autres études ont besoin d'un plus grand nombre de patients dans l'avenir.


## Introduction

Chronic mucocutaneous candidiasis (CMC) is a primary immunodeficiency disease presenting with debilitating, persistent and refractory infections of the skin, nails and mucosal tissues by *Candida* species; in particular *Candida albicans* in the majority of the cases [28]. Several clinical features of CMC have been described, some of which are associated with endocrinopathies or autoimmune diseases, such as hypothyroidism and hypoparathyroidism [4]. Patients with CMC rarely develop disseminated or invasive candidiasis, suggesting a defect in the host defense limited to superficial candidal infections [13,17].

Several reports have shown that the defects are almost exclusively in the cellular branch of the immune system, mainly the specific responses to antigens of *Candida* species [26]. The balance between T helper (Th)1 and Th2 cytokines is important in the initiation of the type of immune response. A Th1 cytokine response is associated with resistance to candidiasis, whereas a Th2 response results in susceptibility to infection [24]. Some CMC patients present serum factors that inhibit the proliferative responses of peripheral blood mononuclear cells (PBMC) from *Candida*-sensitized normal subjects [3]. Durandy et al. [10] demonstrated that infection with *C. albicans* leads to an accumulation of mannan (a *Candida* polysaccharide antigen). This causes activation of suppressor T-cells capable of inhibiting both the proliferation and the activation of T-helper cells.

It is generally accepted that defense mechanisms encompass macrophages, cytotoxic lymphocytes and natural killer (NK) cells. For activation of these cells, pro-inflammatory cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α are major mediators, whereas anti-inflammatory cytokines, such as interleukin (IL)-4 and IL-10, antagonize the cellular anti-candidal defense [23]. Moreover, other recent studies demonstrated deficient production and secretion of IL-2 by PBMC in response to *Candida* antigen [21]. It has also been revealed that other cytokines can influence the balance of cytokines produced, as is the case with IFNγ, which stimulate Th1 responses. Specifically, the pro-inflammatory IL-17—producing Th17 subset is implicated in protection against *Candida* at epithelial surfaces [7].

Due to the several controversial aspects of CMC, we assessed the pro- and anti-inflammatory cytokine responses in a whole-blood culture model after stimulation with *C. albicans* antigens and phytohaemagglutinin (PHA) mitogen in patients with CMC.

## Patients and methods

### Patients and sample collection

The CMC patient group consisted of 13 cases (5 male and 8 female) between 6 and 17 years of age, representing clinical criteria for persistent and refractory candidiasis of skin, nails and mucosal tissues. Thirteen patients presented oral thrush, 5 had nail lesions, 13 trunk skin involvement, 3 scalp skin involvement, 4 oesophageal lesions and 1 conjunctivitis. During the study, the CMC patients did not suffer from other concurrent disorders or acute infections. In all patients, endocrinopathy was excluded as judged by absence of clinical manifestations and laboratory evidence of autoimmune endocrinopathy. The control group consisted of 13 age- and sex-matched healthy individuals. Ethical approval was granted by Ethical Committee of Imam Khomeini Hospital, Tehran, Iran. After obtaining informed consent, blood samples were obtained from both patients and controls at the same time, using 2 ml glass tubes containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ).

### Cell separation and cell cultures

Peripheral blood mononuclear cells (PBMCs) were obtained by centrifugation on Ficoll-Hypaque. The cells were washed twice with RPMI 1640 (Gibco, Grand Island, N.Y.), the number of viable lymphocytes was determined by trypan blue