Impaired T_H17 responses in patients with chronic mucocutaneous candidiasis with and without autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy

Wan-Fai Ng, MRCP, PhD,[a]* Alexei von Delwig, MD, PhD,[a]* Andrew J. Carmichael, MBBS, FRCP,[b] Peter D. Arkwright, MD, PhD,[c] Mario Abinun, MD, PhD,[d] Andrew J. Cant, MD, PhD,[d] Stephen Jolles, MD, PhD,[e] and Desa Lilic, MD, PhD[a] *Newcastle upon Tyne, Middlesbrough, Manchester, and Cardiff, United Kingdom*

Background: Accumulating evidence implicates T_H17 cytokines in protection against *Candida* species infections, but the clinical relevance is not clear. Chronic mucocutaneous candidiasis (CMC) is a heterogeneous syndrome with the unifying feature of selective susceptibility to chronic candidiasis. Different subgroups with distinct clinical features are recognized, including autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED), CMC with hypothyroidism, and isolated CMC. Understanding immune defects in patients with CMC will define cellular and molecular mechanisms crucial for protection against *Candida* species in human subjects.

Objectives: We sought to determine whether impaired T_H17 responses underlie susceptibility to *Candida* species infections and whether the same defect is present in different CMC subgroups.

Methods: We assessed T_H17 responses of PBMCs to *Candida* and non-*Candida* species stimuli by measuring IL-17, IL-22, IL-21, IL-6, IL-23, and IFN-γ cytokine production using cytokine arrays and intracellular cytokine-producing cell numbers and proliferation with flow cytometry. PBMCs from healthy subjects and unaffected family members served as controls.

Results: In patients with CMC with hypothyroidism, T_H17 cells demonstrated decreased proliferation and IL-17 production in response to *Candida* species. In contrast, in patients with APECED, T_H17 cell proliferation and IL-17 production were normal unless exposed to APECED plasma, which inhibited both functions in both APECED and normal PBMCs. *Candida* species–stimulated IL-22 production was impaired in all patients with CMC, whereas IL-6 and IL-23 responses were unaltered.

Conclusion: An impaired T_H17 response to *Candida* species, although mediated by different mechanisms, was present in all CMC subgroups studied and might be a common factor predisposing to chronic candidiasis. (J Allergy Clin Immunol 2010;126:1006-15.)

Key words: Candida *species, chronic mucocutaneous candidiasis, autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy, autoimmune polyendocrinopathy type 1, autoimmune regulator, primary immunodeficiency, T_H17, IL-22, IFN-γ, cytokines*

Candida albicans forms part of the normal flora of the skin and mucosal surfaces and does not cause disease in healthy subjects. However, in patients with chronic mucocutaneous candidiasis (CMC), *Candida* species leads to chronic or recurrent candidiasis of the skin, gut, and other mucosal surfaces. Unraveling the underlying immune defects that predispose patients with CMC to *Candida* species infections would elucidate the pathogenesis of this intriguing primary immune deficiency and further our understanding of the immune pathways that are important in protection against *Candida* species.[1]

It is now clear that CMC is not a single disease but a spectrum of disorders with a common feature of selective susceptibility to *Candida* species infections. Several different subgroups of CMC have been described, including autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED), also known as autoimmune polyendocrinopathy type 1; CMC with hypothyroidism (CMC-ht); and isolated CMC (CMC-i).[1] Whether the same immune defect accounts for the predisposition to *Candida* species infection in these different subgroups is not known.

Data from murine models suggest that T_H1 cytokines are important in the protection against *Candida* species infections.[2] However, our own experience using IFN-γ in a patient with CMC showed a marked reduction in *Candida* species–stimulated IL-6 production *in vitro,* but there was no clinical benefit observed.[3] Furthermore, the observation that patients with IL-12 receptor β1 deficiency and mutations in the IFN-γ receptor signaling pathways are rarely prone to *Candida* species infections has challenged the importance of T_H1 cells in *Candida* species immunity in human subjects.[4] Our own previous findings pointed to dysregulated T_H1 and IL-23 cytokine production.[5-7] More recent data suggest that IL-17 is required for protection in a murine model of systemic candidiasis,[8] whereas *Candida* species–specific memory T cells from healthy volunteers were skewed toward the T_H17 lineage.[9] Patients with the classical hyper-IgE syndrome (HIES) who have chronic candidiasis are known to have

From [a]the Musculoskeletal Research Group, Institute for Cellular Medicine, Newcastle University, Newcastle upon Tyne; [b]the Department of Dermatology, James Cook University Hospital, Middlesbrough; [c]the Department of Child Health, University of Manchester, Royal Manchester Children's Hospital; [d]the Department of Paediatric Immunology, Newcastle General Hospital, Newcastle upon Tyne; and [e]the Department of Medical Biochemistry and Immunology, University Hospital of Wales, Cardiff.

*These authors contributed equally to this work.

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Reprint requests: Desa Lilic, MD, PhD, Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, NE2 4HH, United Kingdom. E-mail: Desa.Lilic@ncl.ac.uk.

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Abbreviations used
AIRE: Autoimmune regulator
APECED: Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy
CFSE: Carboxyfluorescein diacetate succinimidyl
CMC: Chronic mucocutaneous candidiasis
CMC-ht: Chronic mucocutaneous candidiasis with hypothyroidism
CMC-i: Isolated chronic mucocutaneous candidiasis
HIES: Hyper-IgE syndrome
MFI: Median fluorescence intensity
STAT: Signal transducer and activator of transcription

mutations in the gene encoding signal transducer and activator of transcription (STAT) 3, which abrogates early steps in T_H17 differentiation, and these patients have markedly impaired IL-17 production.[10-12] Mutations of caspase recruitment domain-containing protein 9 and polymorphisms of dectin-1 genes associated with impaired IL-17 production were recently reported in families with an unusual susceptibility to mucocutaneous *Candida* species and dermatophyte infections.[13,14] These findings imply that impaired T_H17 responses in human subjects are associated with chronic *Candida* species infections. We and others[15,16] recently reported neutralizing autoantibodies to IL-17A, IL-17F, and IL-22 in patients with APECED, but their effect on T_H17 responses is not fully understood. Low IL-17 production was recently reported in 2 patients with CMC-i,[17] but T_H17 responses have not been assessed in patients with CMC-ht or patients with CMC and APECED.

In this study we investigated T_H17 responses to the C*andida* species microorganism in 18 patients with CMC classified into clinically well-defined CMC subgroups (10 with CMC-ht, 5 with APECED, and 3 with CMC-i), 4 unaffected family members with CMC-ht, and 11 healthy volunteers by measuring T_H17 cytokine production, assessing cellular sources and their proliferating capacity and the effect of patients' plasma. Our results demonstrate IL-17 defects in all CMC subgroups, although mediated by different mechanisms, suggesting that impaired T_H17 responses could be a common factor in patients with CMC being predisposed to chronic *Candida* species infection.

METHODS

Subjects

For more information on subjects, see Table I[15,16,18,19] and the Methods section in this article's Online Repository at www.jacionline.org.

We studied 18 patients with CMC, 5 with APECED and 13 without APECED. All patients had recurrent mucocutaneous *Candida* species infection (mouth, nails, skin, esophagus, and perineum).

All patients were screened for the 2 most common autoimmune regulator (*AIRE*) gene mutations (p.R257X, a nonsense mutation in exon 6, and c.964del13, a 13-bp deletion in exon 8), as well as for autoantibodies to the type 1 interferons IFN-α2 and IFN-ω, IL-17A, IL-17F, and IL-22, which were found in all patients with APECED and none of the patients without APECED and control subjects previously reported.[15,18,19]

All patients were screened for systemic and organ-specific autoantibodies, as well as for thyroid, adrenal, and parathyroid function. A diagnosis of endocrinopathy was based on both clinical and laboratory evidence of endocrine hypofunction.

Patients with APECED. Five patients from 3 families were found to have an *AIRE* gene mutation (c.964del13 deletion) and APECED, Online Mendelian Inheritance in Man (OMIM) 240300 (see additional data in the Methods section of this article's Online Repository).

Patients without APECED. Thirteen patients without APECED but with CMC from 6 families did not have any clinical, laboratory, or genetic signs suggestive of APECED. Ten patients without APECED from 4 kindreds were designated as having CMC-ht, OMIM 114580. None of the patients scored positive for HIES,[20] nor were *STAT3* or *STAT4* gene mutations detected (B. Grimbacher and M. Netea, personal communications). The remaining 3 patients with CMC did not have an associated endocrinopathy and were designated as having CMC-i, which included both dominant and recessive inheritance (see additional data in the Methods section in this article's Online Repository).

Control subjects. Eleven healthy laboratory volunteers were recruited as control subjects for the study. Four relatives of patients with CMC-ht unaffected by candidiasis, hypothyroidism, or both were included as separate control subjects.

Informed consent was obtained from all patients and healthy control subjects. Ethical approval was obtained from the Newcastle and North Tyneside Local Research Ethics Committee.

Antigens and cell culture medium

Candida albicans hyphae were prepared as previously described[7] and used as a stimulus at a final concentration of 1:15,000 (equivalent to 1.1 μg/mL protein or 1.1×10^5 CFU/mL). LPS (100 ng/mL) was obtained from phenol extract of *Salmonella typhimurium* (strain ATCC 7823; Sigma-Aldrich, Gillingham, United Kingdom). Peptidoglycan (10 μg/mL) from *Staphylococcus aureus* was purchased from InvivoGen (Wiltshire, United Kingdom). Culture medium RPMI 1640 containing 3 mmol/L L-glutamine, 50 μmol/L 2-mercaptoethanol, and 30 μg/mL gentamicin (all from Sigma-Aldrich) supplemented with 10% FCS (PAA Laboratories GmbH, Pasching, Austria) was used.

Cell stimulation

PBMCs (1×10^6 cells/mL) from patients with CMC and healthy control subjects were stimulated for 1 day (LPS or *Candida* species) and 9 days (peptidoglycan or *Candida* species), respectively, to test the effect of various stimuli on cytokine production by monocytes and T cells. Supernatants were collected and stored at $-20^{\circ}C$ until use. After 9-day cultures, cells were stimulated with phorbol 12-myristate 13-acetate (50 ng/mL, Sigma-Aldrich) and ionomycin (1 μg/mL, Sigma-Aldrich) for 5 hours, with the last 4 hours in the presence of 5 μg/mL Brefeldin A (Sigma-Aldrich). PBMCs were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE; Sigma-Aldrich) at a final concentration of 1 μmol/mL before setting up the cultures to assess proliferation of IL-17–secreting CD4 T cells. APECED or control PBMCs were cultured in 5% autologous or normal heat-inactivated plasma (1 hour at $56^{\circ}C$ in a water bath) to assess the effect of patients' plasma.

Flow cytometric analysis of intracellular cytokine production

Cells were incubated for 1 day, stained, and analyzed in the monocyte gate to assess IL-6 and IL-23 production; for IL-17 and IFN-γ production, cells were incubated for 9 days, stained, and analyzed in the $CD4^+$ lymphocyte or CD4/CCR4/CCR6/CXCR3 gate. Further details are shown in the Methods section in this article's Online Repository.

Cytokine ELISA

Concentrations of cytokines in culture supernatants were measured by using ELISA-based cytokine kits: IL-17A, IL-23, IL-21 (Ready-Steady-Go Kit and MesoScale Discovery; eBioscience, San Diego, Calif), IL-22 (Emelca Bioscience, Breda, The Netherlands), and IFN-γ (antibody pairs; BD PharMingen) levels were measured according to manufacturer's