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Letter to the Editor

Invariant natural killer T (iNKT) cell deficiency in chronic mucocutaneous candidiasis – A consequence or a cause?

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ABSTRACT

Chronic mucocutaneous candidiasis (CMC) is a group of heterogeneous disorders characterised by primary selective susceptibility to chronic, recurrent *Candida* infections. The genetic defect of one subgroup of CMC patients have been identified as mutations of the autoimmune regulator (*AIRE*) gene. Recent data implicated the *AIRE* gene in iNKT cell development, raising the possibility that iNKT cells may be important in defending against *Candida* infections. In this study, we enumerated the circulating iNKT frequency in 22 CMC patients (9 with *AIRE* gene mutations) and 25 healthy controls. We also examined the effect of *Candida* stimulation on iNKT cells in vitro. Our data demonstrated that peripheral iNKT cell frequency is significantly reduced in CMC patients compared to healthy controls, regardless of their *AIRE* gene mutation status. Direct stimulation with heat-inactivated whole *Candida* did not induce iNKT cell proliferation. Furthermore, circulating iNKT cell frequencies in some healthy controls were comparable to CMC patients. These observations suggest that iNKT cell deficiency is part of the CMC disease phenotype irrespective of the presence of *AIRE* gene mutations but does not appear to confer susceptibility to chronic *Candida* infections. We postulate that the reduced circulating iNKT cell frequency in CMC is a consequence rather than a cause of chronic *Candida* infections.

1. Letter to the Editor

Invariant natural killer T (iNKT) cells are a unique subset of T cells which use a restricted repertoire of T cell receptor α- and β-chains (V_α24-$_\alpha$J281 preferentially paired with V_β11 in humans) and express surface molecules that are usually found on natural killer cells. Unlike conventional T cells, iNKT cells recognise glycolipids presented by major histocompatibility complex class I-like molecules, CD1d. Data from rodent models implicate iNKT cells in the regulation of many immune responses [1] but their role in human diseases remains to be fully defined. Chronic mucocutaneous candidiasis (CMC) is a rare, heterogeneous group of conditions characterised by a primary selective susceptibility to persistent or recurring *Candida* infections [2]. A subset of CMC patients harbour mutations of the autoimmune regulator (*AIRE*) gene and also develop organ-specific autoimmune diseases and ectodermal dystrophy [3], hence termed Autoimmune Poly-Endocrinopathy Candidiasis Ectodermal Dystrophy (APECED) syndrome. Interestingly, Lindh et al recently reported that iNKT cell development was impaired in *aire*$^{-/-}$ mice and in 3 individuals with *AIRE* gene mutations [4]. In contrast, Pitt and colleagues showed that iNKT cell development is unimpaired in *aire*$^{-/-}$ mice [5] and Somech et al. reported that children with Omenn syndrome have reduced transcriptional expression of the *AIRE* gene but normal iNKT cell frequency [6]. The reason for the discrepant findings is not clear. Furthermore, peripheral iNKT cell frequency in CMC patients without *AIRE* gene mutations has not been studied.

We enumerated the circulating iNKT cell frequency in 22 CMC patients including 9 with *AIRE* gene mutations, and 25 healthy controls (Table 1). The two groups were similar in age and gender. None of the CMC patients were taking immuno-modulatory medications at the time of study. iNKT cells were defined by the co-expression of V_α24 and V_β11 on CD3$^+$ lymphocytes using flow cytometry (gating strategy is shown in supplementary Fig. 1). This method has been demonstrated to correspond well with iNKT cell frequency determined by CD1d tetramers or a monoclonal antibody specific for the invariant CDR3 loop of the human canonical V_α24J_α18 chain, even at low numbers [7,8]. The frequency of circulating iNKT cells among CD3$^+$ lymphocytes in CMC patients was significantly lower compared to healthy controls (mean = 0.018 ± 0.003% versus 0.061 ± 0.012%, $p = 0.008$) regardless of the *AIRE* gene mutation status (Fig. 1a and b), indicating that reduced iNKT cell frequency is part of the CMC disease phenotype. Serial measurements over a period of 6–12 months in 5 healthy volunteers and 3 CMC patients (Fig. 1c and d) showed that the iNKT cell frequency was stable over time. The absolute number of circulating iNKT cells was not specifically determined but since observations from routine clinical practice suggest that the lymphocyte counts of CMC patients were usually within normal ranges [9,10], we anticipated that the absolute numbers of peripheral blood iNKT cells among CMC patients were also reduced compared to healthy controls. Consistently, we have estimated the absolute circulating iNKT cell numbers based on the contemporaneous absolute lymphocyte counts of CMC patients and mean reference lymphocyte counts of healthy volunteers, and showed that the absolute numbers of peripheral blood

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Table 1
Numbers and characteristics of CMC patients and healthy controls.

	Total	Gender	Age (mean, range)	AIRE gene mutations[c]	Anti-IFN α and ω antibodies[d]
Patients					
AIRE$^{-/-}$ CMC[a]	9	6M, 3F	20 (3–38)	9/9 c.964del13	9/9 anti α & ω
AIRE$^{+/+}$ CMC[b]	13	6M, 7F	16.2 (2–47)	0	0
All CMC	22	12M, 10F	17.9 (2–47)	9/22	9/22
Healthy controls					
	25	12M, 13F	24.6 (4–61)	ND	ND

Numbers in boxes denote numbers of individuals, gender (M = male, F = female) and positivity for trait out of total number in specified groups.
[a] CMC patients with *AIRE* gene mutations.
[b] CMC patients without *AIRE* gene mutations.
[c] All patients were screened for the two most common *AIRE* gene mutations and c.964del13 (a 13bp deletion in exon 8) and p.R257X (a nonsense mutation in exon 6).
[d] All patients were screened for autoantibodies to type 1 interferons (IFNs) which are selectively found in patients with APECED (Meloni A, Furcas M, Cetani F, et al. Autoantibodies against type I interferons as an additional diagnostic criterion for autoimmune polyendocrine syndrome type I. J Clin Endocrinol Metab 2008;93:4389–97.); numbers denote positivity for autoantibodies to IFNα2 and ω.

iNKT cells were also significant lower among CMC patients than healthy controls (supplementary Fig. 3 and supplementary Tables 1 and 2).

In this study, although the range of iNKT cell frequency among healthy individuals is wide, it is evident that the iNKT cell frequency was comparable between some healthy individuals and CMC patients suggesting that reduced circulating iNKT cell frequency *per se* is perhaps unlikely to confer susceptibility to *Candida* infections.

To further investigate whether iNKT cells may play a role in the protection against *Candida* infections, we stimulated control PBMC with heat-inactivated whole *Candida* and determined whether this induced iNKT cell expansion. As anticipated, stimulation of PBMC with an iNKT cell-specific agonist, α-galactosylceramide, induced marked expansion of iNKT cells after 10–12 days. In contrast, no apparent expansion of iNKT cells was observed in *Candida*-stimulated PBMC culture (Fig. 2a). In addition, co-culture of iNKT cell lines generated from 2 healthy individuals with heat-

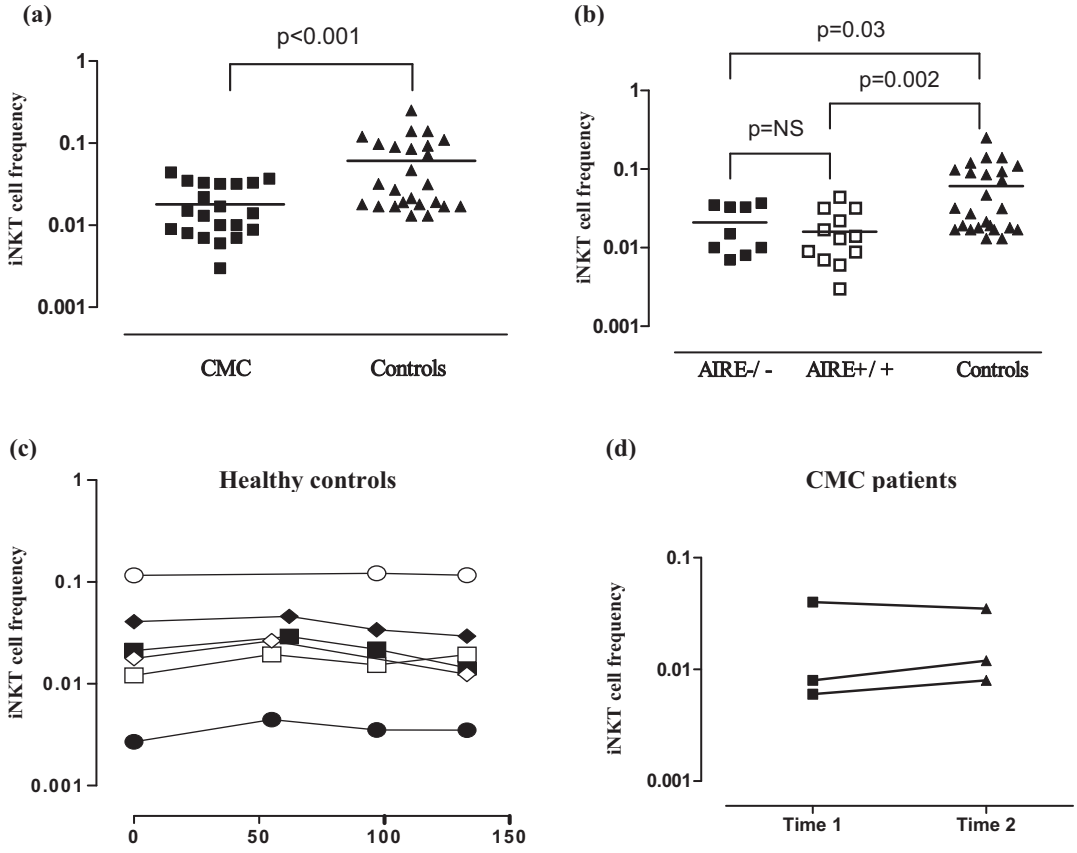

Fig. 1. Circulating iNKT cell frequency of CMC patients is significantly reduced compared to healthy controls and is independent of the AIRE gene mutation status. The frequency of peripheral blood iNKT cells was enumerated using flow cytometry. iNKT cells were identified by the co-expression of $CD3^+V_\alpha14^+V_\beta11^+$ cells. (a) The percentage of iNKT cells among lymphocytes was shown for all CMC patients (CMC, filled squares) and healthy controls (Controls, filled triangles). (b) The percentage of iNKT cells among lymphocytes was shown for CMC patients with AIRE gene mutation ($AIRE^{-/-}$, filled squares), without AIRE gene mutation ($AIRE^{+/+}$, unfilled squares) and healthy controls (Controls, filled triangles). Horizontal lines represent median values of the groups. (c) and (d) Peripheral blood iNKT cell frequency is stable over time. Circulating iNKT cell frequency was measured over a period of 6–12 months in 5 healthy volunteers (c) and 3 CMC patients (d).