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Omenn's syndrome occurring in patients without mutations in recombination activating genes

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Abstract

Omenn syndrome (OS) is characterised by hepatosplenomegaly, lymphadenopathy, erythema, eosinophilia, elevated IgE, oligoclonal T cell expansions and recombinase activating gene (*RAG*) mutations. We investigated 9 cases of OS to correlate genotype with immunophenotype using a two-color flow cytometry with monoclonal antibodies against CD3 and TCRVB families to map TCRVB usage. T and B clonal cell populations were examined in peripheral blood lymphocytes by PCR and sequencing of *TCRB/TCRG* T cell and *IGH* FR2/FR3 B cell products. *RAG* and *Artemis* genes were sequenced from genomic DNA.

All patients demonstrated absent TCRVB families; six had predominant TCRVB families, six oligoclonal *TCR* gene rearrangements including *TCRGD* rearrangements. One demonstrated functional *IGH* rearrangement, an observation not previously reported. In this clinically homogeneous population, with similar immunological phenotype, *RAG* mutations were identified in only 2/9 patients.

OS is a genetically heterogeneous condition, and patients with similar immunophenotypes may have as yet unidentified gene defects.

Keywords: Omenn syndrome; Recombination activating gene; *Artemis* gene; T cell receptor oligoclonality

Introduction

A wide repertoire of T cell receptor or immunoglobulin diversity is achieved by somatic recombination of variable (V), diversity (D) and joining (J) gene segments. The recombination activating gene (RAG)-1 and RAG-2 endonuclease proteins initiate this recombination by cleaving DNA at recombination signal sequence sites. Cleaved DNA is subsequently repaired by the non-lymphoid-specific DNA double-strand break repair machinery [1]. Mutations in *RAG-1* or *RAG-2* genes result in a functional impairment of antigen receptor recombination, which causes T-negative B-negative severe combined immunodeficiency (T-B-SCID) [2].

Omenn syndrome (OS), first described in an extended American-Irish family, is characterised by erythroderma, hepatosplenomegaly and lymphadenopathy with accompanying respiratory and gastrointestinal symptoms and failure to thrive [3]. Other features include raised serum IgE, eosinophilia and hypogammaglobulinemia as well as a T + B-peripheral lymphocyte profile dominated by activated, anergic T cells. Subsequent studies of small numbers of patients have found clonal T cell population expansions with restricted TCRVB family usage [4]. Skin biopsy shows an activated autologous T cell infiltrate. Lymph node architecture is abnormal, typically with an absence of germinal centres and paracortical expansion of S100 staining interdigitating reticulum cells [5].

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The description of a family with OS (T + B-SCID) in 1 sibling and alymphocytosis-type severe combined immunodeficiency (T-B-SCID) in a subsequent sibling suggested that OS may be a "leaky" form of T-B-SCID [6]. Subsequently, missense mutations in *RAG-1* and *RAG-2* were identified in seven patients with OS [7], which severely restricted the function of the recombinase proteins but permitted the expansion of oligoclonal populations of host T cells. In one study of *RAG*-deficient patients, it was concluded that null mutations on both *RAG* alleles lead to the T-B-SCID phenotype whereas patients manifesting classic OS have missense mutations on at least one *RAG* allele and maintain partial V(D)J recombination activity, accounting for the generation of residual, oligoclonal T-lymphocytes [8]. However, both T-B-SCID and OS have been described in 3 different families with identical *RAG-1* or *RAG-2* missense mutations suggesting that the clinical phenotype is not explained by the underlying gene mutation alone [9]. The spectrum of RAG deficiency has expanded to include patients with atypical OS [8]. In this intermediate group of patients, erythroderma and hepatomegaly were present, but not lymphadenopathy. Some lymphocyte proliferation to PHA and presence of B cells were also described associated with RAG gene defects [8].

Recently 5 infants from 4 unrelated families with OS were reported, only 2 of whom had mutations in *RAG-1* [10]. Three other clinically similar patients had *RAG* gene polymorphisms, which do not affect endonuclease function, suggesting that mutations in other genes can give rise to a clinical phenotype similar to OS.

Mutations in the *Artemis* gene also cause T-B-SCID [11] and have been described in patients with features of OS but who had maternofetal engraftment (MFE) [12, 13]. The demonstration of MFE precludes a diagnosis of OS. *Artemis* is involved in opening of the hairpin formed as an intermediate step during V(D)J recombination and thus forms part of the DNA double strand break repair mechanism required to complete V(D)J recombination [14]. More recently, a patient with a defect in *Artemis*, and with features of OS, but no evidence of MFE has been described [15].

In order to correlate gene defect with immunological phenotype, we have analysed a cohort of patients from a single centre, with a clinical diagnosis of OS, for clinical features and lymphocyte oligoclonal expansion, as well as *RAG* and *Artemis* gene defects.

Patients

Nine consecutive patients with a presumptive diagnosis of OS were referred by clinical immunologists to the Northern Supra-Regional Bone Marrow Transplant Unit for SCID at Newcastle upon Tyne, UK, between 1996 and 2001 (Table 1). Clinical diagnostic criteria for OS were as previously described [7], namely the clinical triad of hepatosplenomegaly, lymphadenopathy, erythematous rash in the absence of maternofetal engraftment and presence of either a raised IgE or peripheral eosinophil count, together with immunodeficiency. Maternofetal engraftment was excluded in all cases by electrophoretic separation of radioactive PCR products following amplification of dinucleotide repeat polymorphisms in DNA from separated T cells using standard protocols, or where possible, by cytogenetic analysis. The median age of symptom onset was 3 weeks (range 0–7 weeks). The median age at referral was 10 weeks (range 0–16 weeks). Parental consanguinity was present in 4 families. All

Table 1
Clinical details at presentation of 9 patients with Omenn syndrome

Clinical features	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5[a]	Patient 6	Patient 7	Patient 8[a]	Patient 9
Age at investigation (weeks)	10	10	16	3	10	11	12	birth	11
Sex	F	F	M	F	M	F	F	F	M
Ethnic origin	Caucasian	Caucasian	Caucasian	Asian	Asian	Asian	Caucasian	Asian	Asian
Consanguinity	no	no	no	cousin	cousin	cousin	no	cousin	cousin
Age at onset of rash (weeks)	1	3	6	birth	4	7	1	birth	7
Erythroderma	Generalised, scaly, scalp, face, limbs, trunk	Scalp, face, arms, trunk	Generalised, scaly, scalp, face, limbs, trunk	Generalised, scaly, scalp, face, limbs, trunk	Face, limbs trunk	Generalised exfoliative face, trunk, limbs	Scalp, face, limbs, trunk	Face, limbs, trunk	Extensive thickened skin, face, limbs, trunk
Alopecia	complete	complete	complete	complete	partial	partial	complete	partial	complete
Hepatosplenomegaly (cm)	4/2	4/1	4/4	2/2	2/1	2/0	2/1	4/1	4/2
Features of CHARGE syndrome	no	no	no, 22q11 normal	no	no	no	no	no	no
Infection	nil	Av 41 enteritis	Aspergillus, PCP	nil	BCG	GAS, St aureus	VZV, PCP	nil	CMV

Av 41—adenovirus type 41; PCP—pneumocystis carinii pneumonia; GAS—group A streptococcus; VZV—varicella-zoster; CMV—cytomegalovirus.
[a] Siblings.