Omenn Syndrome in the Context of Other B Cell-Negative Severe Combined Immunodeficiencies

Anna Villa MD, Christina Sobacchi PhD and Paulo Vezzoni MD PhD

Department of Human Genome and Multifactorial Disease, Institute of Biomedical Technology, Medical Research Council, Milan, Italy

Key words: Omenn syndrome, severe combined immunodeficiencies, recombination-activating genes, *Artemis*

Abstract

Severe combined immunodeficiencies represent a heterogeneous group of hereditary defects of the immune system that affect both T and B cells and whose etiology has only recently begun to be understood. A portion of these SCID patients bear a defect in either of the two recombination-activating genes, Rag-1 or Rag-2, while others have mutations in a newly identified gene, *Artemis*. Omenn syndrome is an unusual severe immunodeficiency with T cells but no B cells, and peculiar features also due to a defect in Rag-1 or Rag-2 genes. All these three forms are characterized by an impairment of the VDJ recombination, the process that insures the somatic diversification of immunoglobulin and T cell receptor-encoding genes. Recent findings have enabled us to better understand the pathophysiology of these three immunodeficiencies, which affect the V(D)J recombination process to a different extent and in different ways.

IMAJ 2002;4:218–221

Lymphoid cells are the building blocks of a highly sophisticated system that has evolved to defend organisms against the invasion of foreign agents by distinguishing the self from the non-self. T and B cell lymphocytes are the key players in the adaptive immune system. It is due to these cells that the immune system can recognize non-self-antigens, rapidly reacting with the clonal expansion of a particular subset of cells [1] and with the generation of the immunologic memory [2].

B cells control humoral immunity through antibody production, whereas T cells account for almost all the forms of cell-mediated immunity. This marked difference in function is characterized by the production of different antigen-specific receptors (immunoglobulins for B cells and membrane-bound T cell receptor for T cells), and by the tissues where the development occurs. The earliest recognizable step in B lymphocyte maturation is the rearrangement of genes coding for the immunoglobulins. In brief, the rearrangement of the heavy chain gene of the immunoglobulin defines the pre-B lymphocyte stage, while rearrangement of the light chain gene is a marker of immature B cells, which bear IgM on their surface. T cells, instead, differentiate in the thymus in a well-defined process during which thymocyte precursors divide, rearrange and express T cell receptors. TCRs are assembled similarly to immunoglobulins, and the molecular mechanisms involved are also the same. Pre-T cells are represented by thymic cells positive for both CD4 and CD8, which start rearranging their TCRβ and -α, leading to the production of either CD4 or CD8 single positive cells, which exit the thymus and become competent for their function.

In spite of these differences, the events leading to immunoglobulin and TCR rearrangement are similar in both types of cells and are a prerequisite to the differentiation of both lineages. Indeed, the inactivation of this mechanism completely blocks the differentiation of both B and T lymphocyte in humans and in mice. This fundamental process is called V(D)J recombination, through which genomic fragments are assembled in a single exon that is spliced to a single C segment (Cμ) in immature B cells.

The VDJ recombination process sees the **r**ecombination-**a**ctivating **g**enes Rag1 and Rag2 as the principle actors. Disruption of the Rag-1 or Rag-2 function blocks initiation of V(D)J recombination and leads to the absence of mature B and T cells in mice and humans [2–4]. The V(D)J recombination process can be divided into two stages. In the first stage (cleavage), a double-strand break is produced at the border between the coding element and the heptamer of the recombination signal sequence. This break results in the generation of coding ends and signal ends. In the second phase (joining), the two coding ends join together to form the coding joints, and the two signal ends are ligated, forming the signal joints.

According to the current model [see ref. 5 for complete description of this process], Rag molecules are involved mainly in the first phase of V(D)J recombination. Rag-1 binds to the nonamer element and recruits Rag2. In this way Rag2 makes contact with the heptamer, forming, together with other proteins, a stable complex (Rag-DNA). In the second step, this complex introduces a nick at the 5′ end of the RSS heptamer, leaving a 3′OH terminus on the coding side, and a 5′ phosphate group on the signal side. Finally, the Rag proteins convert the nick into a flush double-strand break, with the formation of a DSB, giving rise to cleavage intermediates, a covalently sealed hairpin coding end, and a 5′ phosphorylated blunt

SCID = severe combined immunodeficiencies
Ig = immunoglobulin

TCR = T cell receptor
RSS = recombination signal sequence
DSB - double-strand break

signal end. However, it is likely that Rags play a role also in the second phase, since they have been involved also in the opening of the hairpin and in coding end processing [6,7].

Mutational analysis has identified a core region of the Rag1 (murine amino acids 384-1008) and Rag2 (murine amino acids 1-371) proteins, which is sufficient for Rag catalytic activity in both cellular and *in vitro* assays [Figure 1]. However, the Rag-1 N terminus (amino acids 1-383) enhances the efficiency of recombination *in vivo* and facilitates the nuclear import [8,9]. Rag1 murine (389-446) contains a region termed the nonamer binding domain that displays homology to the homeodomains of members of the Hin family of bacterial invertases. The nucleolytic activity (catalytic site) is mediated by a region C terminal to the Rag1 NBD, containing the three amino acid residues (D600, D708 and E 962) that form the active site of the VDJ recombinase. Sequence and mutational analyses of Rag2 have shown that the active core (amino acids 1-371) contains six kelch motifs that predict a six-bladed beta-propeller fold [10,11]. Such a configuration suggests a possible regulatory role in coordinating multiple protein-protein interactions. The C-terminus of Rag2 contains a plant homeodomain finger domain spanning a region of about 50–80 amino acids. This motif has been identified in a number of chromatin remodeling proteins. In line with a potential role in chromatin remodeling, this region of Rag2 has been implicated in facilitating the ordered and efficient assembly of V_H and DJ_H antigen receptor segments and the remodeling of the Rag1/Rag2 RSS complex [12].

Upon formation of the stable Rag1/Rag2 RSS complex, DNA cleavage ensues, leading to the production of blunt signal ends and hairpin coding ends. Ubiquitously expressed proteins, including Ku70 and Ku 80, DNA-dependent protein kinase and ligase IV, are required to repair the Rag-mediated breaks.

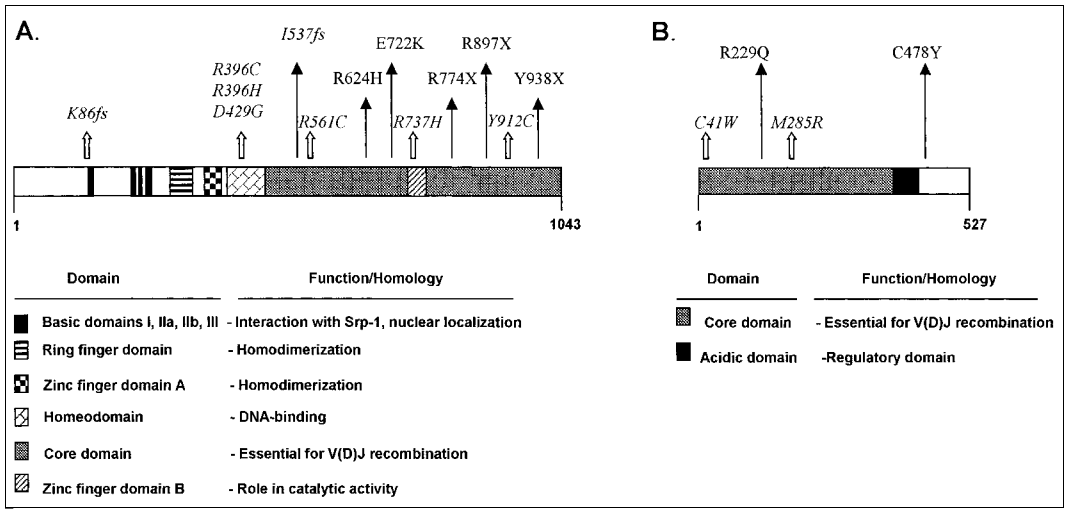

Figure 1. Schematic representation of the full-length human Rag1 **[panel A]** and Rag2 **[panel B]**. Omenn mutations are indicated by white arrows and T-B-SCID mutations by black arrows.

Clinical and immunologic findings of Omenn syndrome and other B-SCID

The fundamental role of Rag genes in lymphoid development was first determined by mouse studies in which Rag1 and Rag2 were inactivated by homologous recombination. However, it took 4 years to demonstrate that these genes were also involved in human pathologies. In 1996, Schwarz and co-workers [2–4] showed that a portion of patients with a clinical picture of SCID with no B and T cells (T-B-NK+SCID) bear severe mutations in Rag1 or Rag2 genes, completely abolishing their activity. As expected, the clinical picture of these patients resembled the Rag knockout mice in which neither T nor B cells are present.

However, mutations in either the Rag1 or Rag2 gene account for only a subset of patients with T-B-SCID [4,13–15]. In our series, Rag-dependent patients account for approximately half of T-B-SCID [15]. Very recently, some patients with typical T-B-SCID, but without Rag abnormalities, have been shown to bear alterations in a novel gene, *Artemis* [1]. Fibroblasts from *Artemis*-deficient patients show an increased radiosensitivity to ionizing radiation [16–18] and a defect in VDJ recombination which is rescued by *Artemis* gene reintroduction. Therefore, *Artemis* encodes for a novel VDJ recombination/DNA repair factor belonging to the metallo-beta-lactamase superfamily, and its investigation is likely to unravel new aspects of the V(D)J recombination process. As *Artemis* maps to chromosome 10p in humans, it is likely that it corresponds to the gene mutated in a T-B-SCID described in native Americans, whose defect has previously been mapped to the same region. An isolated case bearing mutations in Ligase IV gene, also involved in V(D)J recombination, has also been reported [19]. Despite extensive search, no SCID due to defects in Ku, DNA-PK and other DSB DNA repair factors involved in the second phase of V(D)J recombination has been found [17]. At present it is not known whether *Artemis* and Rag-dependent forms cover all the T-B-SCID or whether further heterogeneity is present in this disease.

Interestingly, Rag mutations are also responsible for another immune disease long considered a puzzle for immunologists and pediatricians – Omenn syndrome. In 1965, Gilbert Omenn, then a student at Harvard Medical School, described for the first time a syndrome characterized by reticuloendotheliosis and eosinophilia that occurred in 12 individuals from an extraordinarily inbred Irish-American family [20]. OS is characterized by a complex clinical

NBD = nonamer-binding domain

OS = Omenn syndrome

picture in which repeated infections, indicative of a severe immunologic defect, coexist with a high number of activated, non-functional T lymphocytes. Laboratory findings reveal a characteristic hypereosinophilia and hypogammaglobulinemia, but, paradoxically, IgE serum levels are increased in spite of the absence of detectable B lymphocytes in peripheral blood, skin and lymph node tissues. On the other hand, normal to elevated numbers of autologous, activated T cells with a skewed Th2 profile are present. A *bona fide* diagnosis of Omenn requires that engraftment of maternal T cells be ruled out by human leukocyte antigen typing or molecular analysis.

The analysis of the T cell receptor-beta repertoire showed that the peripheral T cells were oligoclonal [21]. More detailed studies based on sequencing of TCRβ amplified transcripts revealed that these T cell clones have productively rearranged their TCRβ genes with normal, in-frame additions of both P and N nucleotides [22,23]. The restricted TCRβ repertoire in OS patients could arise in the periphery because of a selection-driven expansion of a limited number of clones, despite the existence of a normal unrestricted thymic repertoire. Alternatively, a restricted repertoire could already be present in the thymus due to an inherent inability to efficiently accomplish all the steps required for intrathymic gene rearrangement. The analysis on a deceased patient showed that the TCRβ repertoire is already restricted in the thymus, although further selection occurs in the periphery [23]. These latter observations pointed to a defect in the generation of TCR diversity, raising the possibility that OS is a disease resulting from the impairment but not the complete elimination of the VDJ recombination process.

Molecular findings

The clinical picture of OS is atypical, and *a priori* there were few reasons to suspect that Rag mutations could be responsible for this disease. However, the description of co-occurrences of both OS and SCID within the members of the same family [21] prompted us to investigate these genes. In the original paper we raised the possibility that the differences between SCID and Omenn could be due to the severity of the molecular defect [22]. By analyzing a large series of Rag-dependent immunodeficient patients we were able to further strengthen this hypothesis by showing that, in contrast to the mutations noted in SCID patients, most of the OS patients analyzed so far show at least one allele with missense mutations that, when biochemically tested, maintain partial VDJ activity [15,21].

Although the presence of a mutation partially retaining Rag activity is the first determinant of OS, additional events, such as exposure to pathogens, are also required for a full clinical picture of OS [13,24]. In our series we showed that many Rag-dependent patients exhibit clinical features that do not easily match with T-B-SCID or OS. This group, named "atypical SCID/Omenn," is similar from a molecular point of view to classical OS, since these patients also bear missense mutants [15]. In contrast, classical T-B-SCID cases have severe null mutations.

The existence *in vivo* of missense mutations maintaining partial activity suggested that these mutations could affect specific domains outside the catalytic site. We and others tested this hypothesis using a panel of biochemical assays, which largely confirmed this assumption and were instrumental in defining new domains and even novel aspects of Rag function [25,26].

Conclusions

Mutations in Rag genes account for about half of T-B-SCID patients. Severe mutations in Rag genes lead to a complete arrest of T and B cells, giving rise to classical T-B-SCID, while missense mutations allowing partial activity are at the basis of typical or atypical OS. Rag-dependent SCIDs are not radiosensitive, and patients whose cells show an increased sensitivity to ionizing radiation bear defects in the recently identified gene *Artemis* [1]. Radiosensitivity is so far the only feature distinguishing between Rag- and *Artemis*-dependent cases. A few OS patients do not bear mutations in Rag genes, and further investigations will be needed to identify the gene responsible for these Rag-independent cases. In the meantime, the identification of *Artemis* as a novel actor in the V(D)J recombination process and the detailed analysis of Rag mutations will allow us to better understand the molecular basis of this complex and heterogeneous class of immune disorders.

Acknowledgments. Dr. C. Sobacchi was supported by a fellowship from FIRC. We thank Massimiliano Mirolo for technical assistance. The research was supported by Telethon grant (E.0917).

References

1. Moshous D, Callebaut I, de Chasseval R, et al. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 2001;105:177–86.
2. Shinkai Y, Rathbun G, Lam K-P, et al. Rag2-deficient mice lack mature lymphocytes owing to inability to initiate V(D) rearrangement. *Cell* 1992;68:855–67.
3. Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE. Rag1-deficient mice have no mature B and T lymphocytes. *Cell* 1992;68:869–77.
4. Schwarz K, Gauss GH, Ludwig L, et al. Rag mutations in human B cell-negative SCID. *Science* 1996;274:97–9.
5. Sadosfsky MJ. The RAG proteins in V(D)J recombination: more than just a nuclease. *Nucleic Acids Res* 2001;29:1399–409.
6. Besmer E, Mansilla-Soto J, Cassard S, et al. Hairpin coding end opening is mediated by RAG1 and RAG2 proteins. *Mol Cell* 1998;2:817–28.
7. Santagata S, Besmer E, Villa A, et al. The RAG1/RAG2 complex constitutes a 3' flap endonuclease: implications for junctional diversity in V(D)J and transpositional recombination. *Mol Cell* 1999;4:935–47.
8. Cortes P, Ye ZS, Baltimore D. RAG-1 interacts with the repeated amino acid motif of the human homologue of the yeast protein SRP1. *Proc Natl Acad Sci USA* 1994;91:7633–7.
9. McMahan CJ, Difilippantonio MJ, Rao N, Spanopoulou E, Schatz DG. A basic motif in the N-terminal region of RAG1 enhances V(D)J recombination activity. *Mol Cell Biol* 1997;8:4544–52.
10. Callebaut I, Mornon JP. The VDJ recombination activating protein rag2 consists of a six-bladed propeller and PHD fingerlike domain, as revealed by sequence analysis. *Cell Mol Life Sci* 1998;54:880–91.
11. Gomez CA, Ptaszek LM, Villa A, et al. Mutations in conserved regions of the predicted RAG2 kelch repeats block initiation of V(D)J recombination and result in primary immunodeficiencies. *Mol Cell Biol* 2000;20:5653–64.
12. Kirch SA, Rathbun GA, Oettinger MA. Dual role of RAG2 in V(D)J recombination catalysis and regulation of ordered Ig gene assembly. *EMBO J* 1998;17:4881–6.

13. Corneo B, Moshous D, Gungor T, et al. Identical mutations in RAG1 or RAG2 genes leading to defective V(D)J recombinase activity can cause either T-B-severe combined immune deficiency or Omenn syndrome. *Blood* 2001;97:2772–6.
14. Corneo B, Moshous D, Callebaut I, et al. Three-dimensional clustering of human RAG2 gene mutations in severe combined immune deficiency. *J Biol Chem* 2000;275:12672–5.
15. Villa A, Sobacchi C, Notarangelo LD, et al. V(D)J recombination defects in lymphocytes due to RAG mutations: a severe immunodeficiency with a spectrum of clinical presentations. *Blood* 2001;97:81–8.
16. Cavazzana-Calvo M, Le Deist F, de Saint Basile G, Papadopoulo D, De Villartay JP, Fisher A. Increased radiosensitivity of granulocyte macrophage colony-forming units and skin fibroblasts in human autosomal recessive severe combined immunodeficiency. *J Clin Invest* 1993;91: 1214–18.
17. Nicolas N, Moshous D, Cavazzana-Calvo M, et al. A human severe combined immunodeficiency (SCID) condition with increased sensitivity to ionizing radiations and impaired V(D)J rearrangements defines a new DNA recombination/repair deficiency. *J Exp Med* 1998; 188:627–34.
18. Moshous D, Li L, Chasseval R, et al. A new gene involved in DNA double-strand break repair and V(D)J recombination is located on human chromosome 10p. *Hum Mol Genet* 2000;9:583–8.
19. Riballo E, Doherty AJ, Dai Y, et al. Cellular and biochemical impact of a mutation in DNA ligase IV conferring clinical radiosensitivity. *J Biol Chem* 2001;276:31124–32.
20. Omenn GS. Familial reticuloendotheliosis with eosinophilia. *N Engl J Med* 1965;273:427–32.
21. de Saint-Basile G, Le Deist F, de Villartay JP, et al. Restricted heterogeneity of T lymphocytes in combined immunodeficiency with hypereosinophilia (Omenn's syndrome). *J Clin Invest* 1991;87:1352–9.
22. Villa A, Santagata S, Bozzi F, et al. Partial V(D)J recombination activity leads to Omenn syndrome. *Cell* 1998;93:885–96.
23. Signorini S, Imberti L, Pirovano S, et al. Intrathymic restriction and peripheral expansion of the T-cell repertoire in Omenn syndrome. *Blood* 1999;94:3468–78.
24. Kumaki S, Villa A, Asada H, et al. Identification of anti-herpes simplex virus (HSV) antibody-producing B cells in a patient with an atypical RAG1 immunodeficiency. *Blood* 2001;98:1464–8.
25. Santagata S, Gomez CA, Sobacchi C, et al. N-terminal RAG1 frameshift mutations in Omenn syndrome: internal methionine usage leads to partial V(D)J recombination activity and reveals a fundamental role in vivo for the N-terminal domains. *Proc Natl Acad Sci USA* 2000;97: 14572–7.
26. Noordzij JG, Verkaik NS, Hartwig NG, de Groot R, van Gent DC, van Dongen JJ. N-terminal truncated human RAG1 proteins can direct T-cell receptor but not immunoglobulin gene rearrangements. *Blood* 2000;96:203–9.

Correspondence: Dr. A. Villa, Dept. of Human Genome and Multifactorial Disease, Isituto Tecnologie Biomediche, Consiglio Nazionale delle Ricerche, Segrate, Milano, Italy.
Phone: (39-2) 264-22636
Fax: (39-2) 264-22660
email: villa@itba.mi.cbr.it