Immunodeficiency Disorders

The role of a mutation of the *CXCR4* gene in WHIM syndrome

We investigated the role of a mutation of the *CXCR4* gene in 11-year-old twin sisters with WHIM syndrome. The mutated gene may result in production of the mutant CXCR4 protein causing abnormal apoptosis and migratory function, which are thought to be related to the cause of chronic neutropenia in WHIM syndrome.

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Gorlin *et al*. recently described a new immunodeficiency syndrome, characterized by warts, hypogammaglobulinemia, infection and myelokathexis (chronic neutropenia), the WHIM sydrome.[1] We have shown that a mutation of the *CXCR4* gene is responsible for this syndrome and suggested that abnormal signaling from the CXCR4 receptor may cause the clinical symptoms.[2]

The patients were 11-year-old dizygotic twin sisters with a history of recurrent respiratory infections.[2,3] Family members and relatives were healthy and non-neutropenic with normal IgG levels. The diagnosis of WHIM syndrome was made on the basis of the appearance of warts on their hands and legs, and the finding of hypogammaglobulinemia and myelokathexis. The bone marrow of both patients showed an increased amount of mature neutrophils exhibiting hypersegmented nuclei characteristic of myelokathexis. Both girls had marked leukopenia ($<2\times10^9$/L) with decreased numbers of $CD19^+$ B cells. Both had profound neutropenia ($< 0.4\times10^9$/L) and global hypogammaglobulinemia (< 6 g/L).

The primers for DNA amplification of exons 1 and 2 were sense CXCR4-1 and antisense CXCR4-7 or sense CXCR4-2 and antisense CXCR4-6, respectively (Figure 1C). Reverse transcription polymerase chain reaction (RT-PCR) was also performed using the sense CXCR4-5 and antisense CXCR4-6 primer set (Figure 1C). F-actin polymerization and CXCR4 expression was examined using neutrophils incubated for 16 h and mononuclear cells incubated for 24 h with transforming growth factor-β1 (TGF-β1).[4,5] Anti-CXCR4 immunoprecipitates were prepared as previously described.[6] The number of apoptotic cells was evaluated by using an annexin V-fluorescein isothiocyanate apoptosis detection kit. We identified a nonsense mutation truncating the *CXCR4* C-terminal cytoplasmic tail domain, which was a C to T transition at nucleotide position 1000 (R334X) of the *CXCR4* gene in both patients (Figure 1). They were heterozygous for this mutation. Sequencing of the RT-PCR products also disclosed the same mutation (R334X) in one allele in the patients (Figure 1).

Intracellular and surface expression of the CXCR4 protein in neutrophils and CD3 lymphocytes was positive in both the patients and controls (*data not shown*). Immunoblotting analysis showed that the CXCR4 protein in both patients had a molecular weight of 27 kDa, which was slightly lower than that of the wild type CXCR4 (Figure 2A). The observations that CXCR4 protein was reported to be constitutive oligomers[7] and that two different forms of mRNA were found in the patients' leukocytes gave rise to the expectation that two different proteins would appear in immunoblotting. However, we found only one mutant band. This may because of a preferential binding of the antibody to the mutant protein or be due to some other unknown mechanism.

Stromal cell-derived factor-1 (SDF-1) caused significant dose-dependent inhibition of apoptosis of the patients' neutrophils (Figure 2B), whereas CD3 lymphocyte apoptosis was not inhibited (*data not shown*). F-actin polymerization of cultured neutrophils fom the patients was faster than that of neutrophils from controls (Figure 2C). The F-actin polymerization response of CD3 lymphocytes from the patients was slightly higher than that in the corresponding cells from controls (*data not shown*). There was no significant difference in F-actin polymerization of CD3 lymphocytes treated with TGF-β1 between the patients and controls (*data not shown*). We did not compare this function between TGF-β1 treated and non-treated CD3 lymphocytes. If the hyperresponsiveness of F-actin polymerization occurring in WHIM patients were to be corrected by TGF-β1 treatment, this factor could be expected to have a clinical benefit. However, further detailed study will be needed to determine whether TGF-β1 treatment of WHIM is effective or not.

A *CXCR4* knockout mouse, which does not express of CXCR4, exhibits characteristics of hypomyelopoiesis,[8] and some WHIM patients have been reported to have

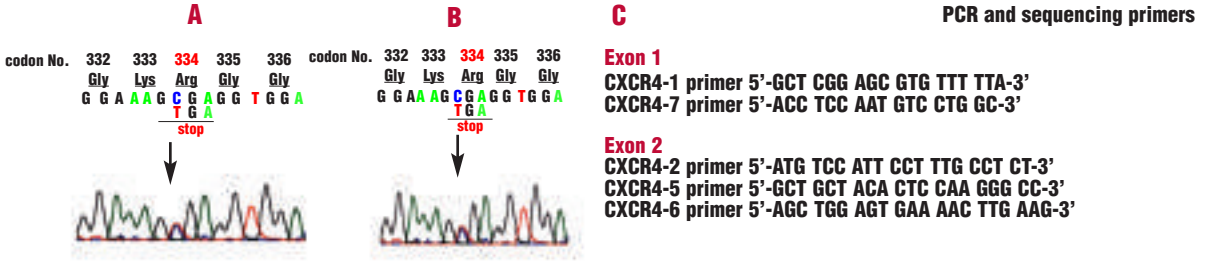

Figure 1. Mutation analysis of the *CXCR4* gene prepared from leukocytes in twin sisters with WHIM syndrome. A nonsense mutation (C→T) truncating the *CXCR4* C-terminal cytoplasmic tail domain occurred at nucleotide position 1000(R334X) of the *CXCR4* gene in one allele of patient 1 (A) and patient 2. Sequencing of RT-PCR products disclosed the same mutation (R334X) in one allele of patient 1 (B) and patient 2.

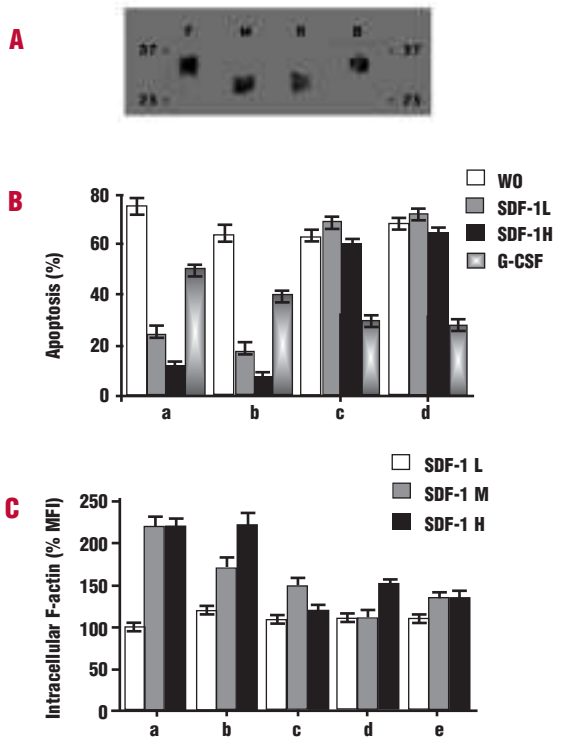

Figure 2. A. Anti-CXCR4 immunoblotting analysis. Anti-CXCR4 (12G5: DakoCytomation, Glostrup, Denmark) immunoprecipitates prepared from leukocytes (1×10^6 from patients) 1 (M) or 2 (R), control 1 (F) or 2 (B) were analyzed by SDS-PAGE and transferred to nitrocellulose. CXCR4 was detected by immunoblotting with an anti-CXCR4 monoclonal antibody (44716: Dako). Mr: relative molecular mass $\times10^{-3}$. Results were reproducible in two separate experiments. **B.** Effect of SDF-1 on neutrophil apoptosis. Neutrophils were cultured with or without SDF-1 (H: 200 ng/mL, L: 20 ng/mL) for 16 h and stained with an annexin V kit. The data shown are representative of three independent experiments: (a) patient 1, (b) patient 2, (c) age-matched control 1, and (d) age-matched control 2. **C.** Effect of SDF-1 on F-actin polymerization of cultured neutrophils. Neutrophils were cultured for 16 h and then exposed (for 2 min) to SDF-1 (H:200 ng/mL, M: 2 ng/mL, L: 0.02 ng/mL), then fixed and permeabilized and stained with FITC-phalloidin (2 μg/mL) for flow cytometric analysis. The proportion of redistributed F-actin in untreated cultured neutrophils as determined by intracellular staining of the cells with FITC-phalloidin was considered to be 100%. The data shown are representative of three independent experiments: (a) patient 1, (b) patient 2, (c) age-matched control 1, (d) age-matched control 2, and (e) age-matched control 3.

myelodysplasia and B-lymphoma.[9] We infer from this information that the interaction between SDF-1 and CXCR4 is related to apoptotic and survival signaling in myelopoiesis. Mature bone marrow neutrophils that survive without undergoing apoptosis may result in increased numbers of mature neutrophils and hypersegmented neutrophils in the patients' bone marrow. In fact, although these mature bone marrow neutrophils are destined to be released from the bone marrow, the migratory hyperresponsiveness of surviving and mature bone marrow neutrophils to SDF-1 in WHIM patients may cause pathological retention of these cells in the marrow compartment. Balabanian *et al*. proposed that CXCR4 1013/CXCR4 wt hetero-oligomers exist in WHIM1013 leukocytes, thereby permitting CXCR41013 to hijack CXCR4wt functioning by a transdominant mechanism, based on an experiment in which the CXCR4 receptor internalization response was shown to be refractory to CXCL12 ligand.[10] The CXCR4 1000/CXCR4 wt hetero-oligomer in our patients may similarly cause abnormal F-actin polymerization and an anti-apoptotic effect as a trans dominant mechanism through G-protein signaling, or the CXCR4 1000 monomer could independently induce these phenomena.

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