
Response:

Intricacies of initiator polymorphism studies

I appreciate Dr Kozak setting the record straight on technical pitfalls and interpretation of studies on initiation of transcription related to polymorphisms close to the initiator methionine. Her detailed critique should be borne in mind while considering the annexin V polymorphism study of Gonzalez-Conejero et al and related studies.

Ellis Neufeld

To the editor:

Polymorphic expression of CD158k/p140/KIR3DL2 in Sézary patients

Sézary syndrome (SS) is a leukemic form of epidermotropic cutaneous T-cell lymphoma (CTCL) characterized by the rapid onset of a pruriginous erythroderma with diffuse adenopathies. Sézary cells are lymphocytes with a typical cerebriform nucleus and a CD4+CD45RO+ phenotype. No specific cell membrane receptor has been described until now. Recently, we reported that circulating and cutaneous Sézary cells express CD158k/p140-KIR3DL2. This transmembrane receptor is a member of the killer cell Ig-like receptors that inhibit natural killer (NK)–mediated lysis after interaction with HLA-A. In healthy individuals, KIR3DL2 is only detected on minor NKs and CD3+CD8+ subsets. Until now, 9 KIR3DL2 alleles have been defined. We reported the restricted expression of the KIR3DL2 008 allele from 2/2 CTCL lines derived from Sézary patients, suggesting that expression of this allelic form could be associated to CTCL malignancies. The role of KIR3DL2 on CTCL cells is still unknown. It could be possible that the KIR3DL2 polymorphism might influence the recognition of HLA-I alleles. In order to determine whether Sézary cells express a specific KIR3DL2 allelic form, we have determined the repertoire of KIR3DL2 expression in 14 patients. Diagnosis of Sézary syndrome was based on clinical criteria (erythroderma, pruritus, palmoplantar keratoderma, diffuse adenopathies) and biologic criteria, including typical circulating Sézary cells (> 1000 cells/μL), histologic data (cutaneous epidermotropic T-cell lymphoma), and detection of an identical T-cell clone in the blood and in the skin by qualitative polychain reaction (PCR)–denaturing gradient gel electrophoresis (DGGE) γ.

Peripheral blood lymphocytes (PBLs) were isolated from heparinized venous blood samples by centrifugation over Ficoll-Hypaque (Eurobio, Paris, France). DNA was extracted using Dneasy tissue kit (Quiagen, Valencia, CA). Three micrograms DNA was PCR-amplified through 40 cycles, with 2 different conditions depending on the KIR3DL2-specific primers used as described. Taq polymerase from Promega (Madison, WI), dNTP 10 mM each (Boehringer Mannheim, Roche Diagnostics, Meylan, France) and 25 mM Mg2+. The amplification products were analyzed on a 1% agarose gel.

The results indicate that in all patients KIR3DL2 CDNA PCR amplification gave good bands. Ten of 14 patients expressed 2 different alleles, including 009/007 (1 patient), 002/005 (1 patient), 007/006 (1 patient), 002/007 (3 patients), 003/007 (3 patients), and 005/007 (1 patient), whereas 4 of 14 patients were homozygous including 009 (2 patients), 002 (1 patient), and 001 (1 patient) alleles. None of the patients was homozygous for KIR3DL2 008 found expressed in CTCL line. These results demonstrate that there is no preferential allelic expression of KIR3DL2 associated with Sézary syndrome.

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References