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Short Report

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Individual CDR3 Regions as Targets for Detection of Clonal Cells in Acute Lymphocytic Leukemia

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Immunoglobulin heavy chain rearrangements provide a specific identity of complementarity determining region 3 (CDR3) DNA sequences. For this reason, CDR3 regions may be used in the diagnosis of malign developments of B lymphocytic cells as a clonal marker (1-4). This study relies on the basis of cloning of the structures belonging to minimal residual disease (MRD) in acute lymphocytic leukemia (ALL) relapses. Experiments in this study were performed in the University of Helsinki Transplantation Laboratory. Samples used with diagnostic purpose were obtained from bone marrow aspiration materials of three patients who had been diagnosed with ALL. The first patient (code: B-ALL 1) was diagnosed with B cell-ALL and classified as L2 according to the FAB classification and his cell surface antigens were determined to be CD 10,19. The second patient (code: B-ALL 2) was diagnosed with B cell-ALL and his cell surface antigens were determined to be CD 10,7,3,8,TdT. The third patient (code: T-ALL) was diagnosed with T-cell ALL and it was determined that the had CD19 as cell surface antigens. DNA samples were isolated using the standard phenol-chloroform extraction method. Amplifications were performed by following consensus primer sequences of the CDR3 region (V (670) Sal CTGTCGACA CGGCCGTGTATTACTG 3' FR3 V, J (36) Pst AACTGCAGAGGAGACG GTGACC 3' J Ig heavy chain). PCR was performed by the Hot-Start PCR method by pre-heating at 96 °C for 5 min. Encoded reaction steps were as follows: (1) 95 °C for 60, (2) 58 °C for 60 s, (3) 72 °C for 90 s. Amplified DNA products were cloned using plasmid PUC 19 and *E. coli* colonies. The cloned product was purified with Qiagen (Germany) reagent. Then the sequencing procedure was performed with an automatic sequencing device (apl. Bios-Mod. 373 A version 2.015). Necessary sequences were determined by consensus primers in all three patients. In a patient with T-cell ALL, specific rearrangements will develop but not a recombinational arrangement directed to VDJ sequences. Therefore, the sequences of the immunoglobulin heavy chain region will remain as information only in the germ line. As a consequence, contrary to the amplification result anticipated from a patient with B-cell ALL, what is expected is that the patient with T-cell would not increase the amplification product on a PCR conducte under the same conditions. The results were in harmony with expectations and only the copy products of consensus primers of patients with B-cell were obtained. It was observed that a cloned product with a length of 140 bp developed in B-ALL 1. Furthermore, in the second patient with B-ALL 2, the existence of one more region which was quite long and identified by the primers was observed as a cloned product. In other words, our first sample was monoclonal and the second one was polyclonal. At the same time, a negative cloning result was obtained in the patient with T-cell, as expected (Figure 1).

Our aim was to indicate the availability of these mapped sequences as an advanced diagnostic monitoring method. Products which were copied from the patient with B-cell leukemia (B-ALL 1) were cloned through plasmids, and isolated after being purified. Patient-specific regions were established from the sequencing

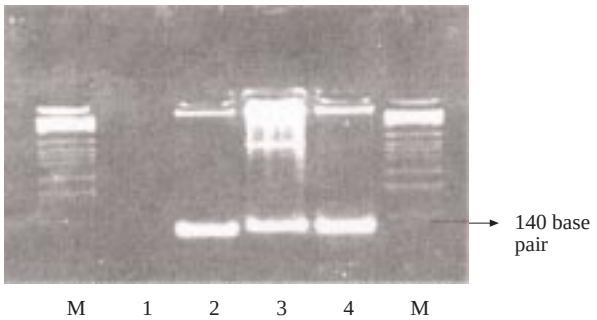

Figure 1. Amplification results of three ALL patients using consensus primers of CDR3 region. (Lane 1, T ALL; Lanes 2 and 4, B-ALL 1; Lane 3, B-ALL 2; M, Marker (PBR 328/Hinf 1).

sample. These regions were synthesized as oligonucleotide nested primers. Only the sequences which indicated patient-specific clonal variance might be used as an amplification sign in relapses and post-transplantation samples. The synthesized nested primers which had high sensitivity to detect MRD structures in further controls were entered in PCR together with bone marrow or blood DNA samples from different periods, which were obtained from pre- and post- bone marrow transplantation cells. An amplification product of expected length was found in all samples (Figure 2). Findings obtained through nested primers in our experiments are in harmony with the results of hybridization studies of Billadeu et al. through allele specific oligonucleotides from ALL, CLL and MM patients and the results obtained by Yamada et al. through oligonucleotide probes on ALL patients (5-6). Some researchers have pointed out the benefits of adding another sign to the immunoglobulin heavy chain in PCR based ALL-MRD detection studies. Hence, it is possible to observe a decrease in false negative results obtained on MRD structures. Despite being expensive and requiring a great deal of labour, these studies indicate the value of using more than one sign. Consequently, it is clear that important gains will be acquired regarding the sensitivity despite an increase in cost, when studies similar to ours are conducted by using more than one oligonucleotide consensus primer (7). Clinical requests underline the importance of the development of purification methods for autologous transplantations while detecting leukemic clones. The disadvantage of both combined cloning and

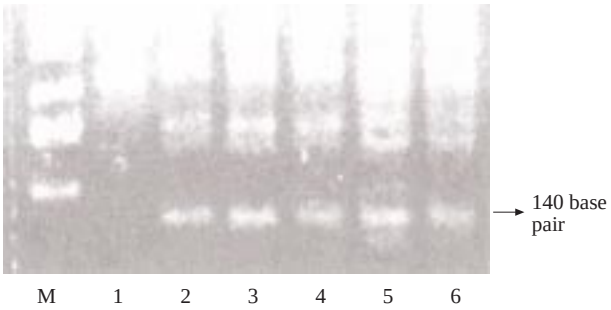

Figure 2. Amplification results of B-ALL 1 patient using patient-specific primers of CDR3 region. (M, Marker (PBR 328 / Hinf 1); Lane 1, control - foreign DNA template; Lane 2, peripheric blood DNA sample in primer diagnosis; Lane 3, bone marrow DNA sample in primer diagnosis; Lane 4, peripheric blood DNA sample in remission after second chemotherapy; Lane 5, harvested stem cells DNA sample before bone marrow transplantation; Lane 6, bone marrow DNA sample in relapse after bone marrow transplantation).

PCR studies based on the CDR3 region is the long time period and cost required for application. Amplification, cloning, purification and sequencing studies with consensus primers necessitate a special laboratory regarding personnel and order.

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