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HLA-B27 DNA Typing using Group Specific Polymerase Chain Reaction

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Abstract: HLA-B27 gene, one of the HLA-class I molecule, is strongly associated with ankylosing spondylitis. It has been most frequently used as a disease-correlated HLA gene by clinicians. In most laboratories, conventional HLA-B27 typing is still performed by cell cytotoxicity tests or fluorescence serology with specific antibodies. In this study, DNA typing method for HLA-B27 was developed by using group specific Polymerase Chain Reaction (PCR). Four HLA-B27 cell lines (HOM-2, JESTHOM, WT24 and BTB) and fifty six B27 Korean individuals defined by serology were used. The results of control cell and B-27 positive individual samples were correlated well with the data which was performed by serological method. All of B27 positive PCR products gave positive signals on Southern blot hybridization with B27 specific probe. This study shows that the HLA-B27 DNA typing is a relatively simple, fast and practical tool for the determination of the HLA-B27 gene in routine clinical laboratory work.
Key Words: HLA-B27, DNA typing, PCR.

INTRODUCTION

HLA (Human Leukocyte Antigen) class I molecules include HLA-A, -B, and -C proteins, which are highly polymorphic glycoproteins expressed on the surface of most nucleated cells[5]. These polymorphic HLA class I molecules bind to foreign or self antigenic peptides and present them to antigen-specific T cells in a self-restricted fashion[32,35]. The HLA genes, predominantly located on chromosome 6, show a high degree of allelic polymorphism [4]. In polymorphic HLA class I genes, the HLA-B is the most polymorphic gene with over 60 alleles characterized so far[12]. Among several HLA allotypes linked to various diseases, HLA-B27 is the most frequently sought after disease-associated HLA genes. This allele is strongly associated with occurrence of several rheumatic diseases, such as ankylosing spondylitis, Reiter syndrome, and acute anterior uveitis[30]. Routine conventional HLA-B27 typing in most laboratories is still performed by serological typing[24] or fluorescence serology with specific antibodies - flow cytometry[1,36]. The advancement of DNA sequencing techniques has shown that the polymorphism of HLA genes is much greater than can be assessed by serology. Thus, serological and cellular typing methods of HLA-B

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27 have been replaced by genetic typing using polymerase chain reaction (PCR)[6-8,13,27,34]. In this experiment, the sequence specific primer pairs were designed corresponding to the serologically defined specificities for identifying the HLA-B27. The aim of our study was to test the feasibility and reliablity of the DNA typing of HLA-B27 using PCR.

MATERIALS AND METHODS

Materials

Four HLA B-27 homozygous cell lines (HOM-2, JESTHOM, WT24 and BTB) used for verifying primer specificity were obtained from the Xth International Histocompatibility Workshop[17]. Serologically defined HLA-B27 positive individual samples (n=56) were anlayzed by DNA typing using PCR.

Serological typing of HLA-B27 was perfomed using the lymphocyte microcytotoxicity assay by standard techniques according to American Society for Histocompatibility and Immunogenetics Manual[16].

Synthesis of Primers

Primers were designed using the nucleic acid sequence of the HLA-B27 gene published by Zemmour and Parham (1992)[40]. Amplification primers were synthesized using a 392 DNA synthesizer and purified by an oligonucleotide purification cartridge (Applied Biosystem, U.S.A.).

HLA-B27 group specific primers, HB1 5'-GCTACGTGGACGACACGCT (codon 148-167) and HB2 5'-CTCGGTCATCTGTGCCTT (codon 280-299) were used for PCR. As an internal control for amplification, 0.1μmol/L of β-globulin primers, GL1 5'- GAAGAGCC AAGGACAGGTAC and GL2 5'-CAACTTCA TCCACGT-TCACC, were used[9].

Extraction and Amplification of DNA

High molecular weight DNA was isolated from peripheral blood leukocytes by phenol/chloroform extraction and ethanol precipitation [18]. Primer mixtures, containing all the ingredients except DNA and *Taq* DNA polymerase, were prepared in 100μl batches sufficient for 10 typings. Primer mix(8μl) was added with 1μl (80 ng) of DNA and 1μl of diluted Ampli*Taq* DNA polymerase (0.25 unit in 1x PCR buffer). The primer mixtures contained 0.4μM of primers, 56mM of KCl, 1.7mM of $MgCl_2$, 11mM of Tris HCl (pH 8.3), 0.0011% (w/v) of gelatin and 250μM each of dNTPs. PCR mixtures were subjected to 35 cycles for 1 min at 94℃, 1 min 30 sec at 70℃, 1 min at 72℃ using an automated thermal cycler (GeneAmp PCR system 9600. Perkin Elmer Cetus Inc.). After addition of 2μl loading buffer (30% (v/v) glycerol with bromophenol blue and xylene cyanol), the amplified DNA fragments were loaded in 2% agarose gel in a glass plate apparatus (Bio Rad Co. Ltd). Gels were examined under UV illumination and documented by photography[23]. The sizes of amplified DNA were compared with the PhiX174/*Hae*III fragments which are especially suitable for sizing linear double-stranded fragments of 152 bp (HLA-B27) and 268 bp (internal control of β-globulin gene).

Southern Blot Analysis of Amplified PCR products

Southern blotting of PCR-products onto nylon membranes (Hybond N+, Amersham, Little Chalfont, U. K.) was carried out according to the instruction protocol (Tropix, Inc. Bedford, Massachusetts, U.S.A). HLA-B27 probe sequences were HB3 5'-GTGGACG A-CACGCGGTTCGTT and HB4 5'-TCATCTG TGCCTTGGTCTT. Nylon membrane was hybridized with 5'-biotinylatd DNA probes (50 ng/mL) using the non-radioactive random labelling system[33]. Luminography was performed by adding chemiluminescent substrate solution to the membrane and exposed for 30 min in the presence of X-ray sensitive film[11].

RESULTS

The results of DNA typing using group specific PCR of four B27 control cells were consistant with the previous report of Kimura et al (1992)[17] using serological method. All of serologically B-27 positive 56 inidividual samples were correctly identified by DNA typing. The reaction efficiency was demonstrated in negative samples by simultaneous amplification of β-globulin gene fragments. HLA-B27 positive samples gave both PCR products of B27 (152 bp) and β-globulin (268 bp) gene fragments, whereas B27 negative samples gave only the β-globulin fragment (Fig. 1A). To confirm the amplification specificity of the PCR, the PCR products were hybridized with HLA-B27 specific oligonucleotide probe. All of HLA-B27 PCR positive samples represented specific DNA band by Southern blot analysis (Fig. 1B). Both the PCR products by agarose gel electrophoresis and the hybridization signals were easily interpretable.

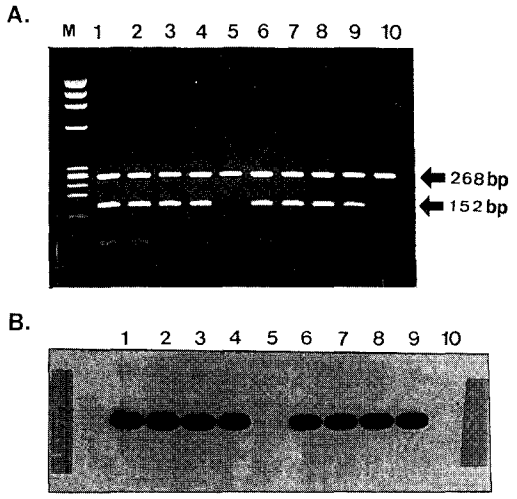

Fig. 1(A). Amplified products of HLA-B27 using PCR analysis Lane M: Molecular weight size marker(PhiX174/*Hae*III), Lane 1: PCR positive control(Cell name: HOM-2), Lane 2,3,4,6,7,8,9: PCR positive samples, Lane 5,10: PCR negative samples
(B). Southern blot analysis for the smaples of 1 (A).

DISCUSSION

HLA-B27 containing 8 different alleles is expressed over 90% of ankyolsing spondylitis whose main symptoms are caused by arthritis of the sacroiliac joints[3,31]. The HLA-B27 gene has been known to be strongly associated with different spondyloarthropathies as well as transplantation immunity. The frequency of HLA-B27 in patients with ankylosing spondylitis is ten times higher than that in normal healthy person[39]. The expression of this gene means a important implication of prognosis in early stage of ankyolsing spondylitis[28]. The traditional serological typing of HLA-B27 involves the use of microcytotoxicity assay of human alloantisera containing specific alloantibodies[2]. Flow cytometry and biochemical techniques have also been used to detect HLA-B27 gene[15].

In recent years, PCR based DNA typing method are becoming more widely used as an alternative to serologic typing[10,14,29]. Authors have reported the DNA typing of HLA-DQA1 and HLA-DQB1 genes using PCR-RFLP (Restriction Fragment Length polymorphism)[19, 20] and HLA-DRB1 gene using PCR-SSP (Sequence specific primers)[21]. The value of the PCR based DNA typing method for the detection of HLA-B27 can be summarized as follows. (i) 1-2 mL of blood is sufficient for PCR reaction. In serologic analysis, however, 10-15 mL of fresh heparinized blood is required. (ii) ambiguity of positive or negative result, which is often encountered in cytotoxicity, is reduced considerably in PCR[37]. (iii) blood samples for PCR can be delivered to the laboratory at any time of the day and stored at 4°C for several days before the DNA is isolated; with serological test, samples have to be prepared as soon as they arrive. (iv) serological method sometimes presents antigen masking when the patient has a small number of lymphocytes or B27 antigens[25].

The risk of PCR-induced contamination, however, requires intense precautions[22,26], including separate rooms for reagent preparation, sample preparation, template addition and PCR analysis[38]. As a conquence, HLA-B27 DNA typing using group specific PCR amplification is a simple, rapid and accurate technique and can be substituted for serological typing in routine clinical work.

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=국문초록=

중합효소연쇄반응을 이용한 HLA-B27 유전자분석

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HLA-class I 항원의 HLA-B 유전자좌에 존재하는 HLA-B27 유전자는 임상적으로 강직성 척수염과 강한 관련성이 있음이 보고되고 있으며, 현재 HLA 유전자중 질병과의 관련성을 보기 위한 검사로 임상에서 가장 널리 사용되고 있다. 대부분의 검사실에서는 현재까지 혈청학적 검사방법을 이용하여 HLA-B27 검사를 실시하고 있는데, 이 방법은 시약이 고가이고, 검체의 안정성과 보관이 어려우며, 분석시간이 오래 걸리는 등 불편한 점이 있고, 또한 현재에도 계속 새로운 HLA-B27 대립유전자가 발견되고 있으므로 위음성의 가능성도 배제할 수 없어, 보다 정확한 검사방법이 요구되고 있다. 최근 HLA-B27 대립유전자의 염기배열이 대부분 밝혀져 혈청학적 방법 대신 DNA를 이용한 typing 방법이 보고되고 있다. 저자들은 HLA-B27 대립유전자에 공통으로 존재하는 염기배열 부분을 선택하여 group specific PCR (Polymerase Chain Reaction)을 실시하고 그 유용성을 검토하였다. 혈청학적 방법으로 HLA B-27 형이 확인된 검체 56 개와 4 개의 표준세포주 (HOM-2, JESTHOM, WT24, BTB)를 이용하여 혈청학적 방법과 DNA typing을 비교한 결과, 두 방법사이에 완벽한 일치를 나타내었다. 따라서 group specific PCR을 이용한 HLA-B27 DNA typing은 검체 및 시약의 안정성이 높고, 경제적이며 신속한 검사가 가능하므로 임상에서 활용성이 매우 클 것으로 사료된다.

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