Rapid Detection of Cutaneous Herpes Simplex Virus Infection with the Polymerase Chain Reaction

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A simple and specific method for detecting herpes simplex virus infection in routinely processed paraffin-embedded biopsy specimens is described. DNA is extracted from paraffin blocks, and subjected to DNA amplification with the polymerase chain reaction. After 40 rounds, an amplified band can be detected after agarose gel electrophoresis and ethidium bromide staining. This band is specific for herpes simplex virus, because tissues infected with related viruses do not give this amplified band. We have been able to detect viral DNA in small punch skin biopsies with this procedure, which can take as little as 6 h. J Invest Dermatol 82:391–392, 1989

The rapid and accurate diagnosis of herpes simplex virus (HSV) infection has become increasingly important in recent years, with the advent of specific therapies and the rising incidence of immunodeficiency states [1]. Towards this end, many new assays have been developed [1]. However, none are entirely satisfactory in terms of sensitivity, specificity, and ease of application. For example, high sensitivity DNA hybridization involves the use of radioactive isotopes and is labor intensive, while immunofluorescence methods require a fluorescent microscope and are not of high sensitivity. Furthermore, these methods usually require the availability of fresh specimens or swabs. This could preclude a specific diagnosis if a herpetic infection was not suspected clinically.

Recently, the polymerase chain reaction (PCR) for selectively amplifying a single DNA segment in a complex mixture was developed [2,3]. Briefly, the sample is denatured and annealed to two oligonucleotide primers which bracket the DNA area of interest. A heat-stable DNA polymerase is added to elongate the primers. After multiple rounds of denaturation, annealing, and elongation, there is selective synthesis of a DNA fragment, corresponding to the target DNA bracketed by the two primers. This fragment can then be detected after gel electrophoresis and ethidium bromide staining [3]. Because of the exponential nature of the amplification, this method can detect extremely small (sub-picogram) amounts of target DNA.

Because we and others have shown that DNA can be extracted from paraffin-embedded tissues [4,5], we sought to use PCR and HSV-specific primers as a rapid method of identifying HSV infection in small biopsies, such as from the skin. Here we present data showing that this is indeed possible, and that there is no cross-amplification of DNA from related human Herpes-viridae. Therefore, this method has the potential of being a clinically useful way of confirming HSV infection.

MATERIALS AND METHODS

Selection of Material Paraffin-embedded blocks of skin biopsies were selected from the archives of the Department of Pathology, University of California, San Francisco. All blocks of tissues with "classic" herpetic histology were used, as well as some blocks showing cellular changes highly suggestive but not diagnostic of herpes infection (i.e., nuclei with cleared-out chromatin and prominent eosinophilic bodies which could represent either large nuclei or viral inclusions, with mixed inflammatory response and/or necrosis). All of the cases had clinical signs and symptoms consistent with herpes simplex infection. As control, three blocks of "tips" from excisions of basal cell carcinomas and one block with culture proven varicella were used. The histology of hematoxylin-eosin stained sections was reviewed by two of us (BE and TD), and the blocks given to another two (MC and XX) "blind" for processing. After PCR was performed on DNA extracted from these blocks, the results were interpreted by the fifth author (TBY) "blind", and then correlated with the previous histologic interpretations.

DNA Extraction DNA was routinely purified from paraffin blocks as described previously [4]. Briefly, the tissue was minced with a razor blade, treated extensively with proteinase K and SDS, and extracted with phenol and chloroform. The DNA was precipitated with ethanol, dried, and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

For rapid DNA extraction, the direct boiling method of Shibata, Arneheim, and Martin [5] was used. Briefly, a 100-µm section was cut from each block, deparaffinized with xylene and ethanol, dried, and boiled for 15 min in 100 µl of TE buffer. After 5 min of centrifugation at 10,000 × g, the supernatant was withdrawn from the pellet material and used for the PCR.

DNA Amplification To amplify HSV DNA using PCR, a pair of primers (Research Genetics, Huntsville, AL) that bracket a 92 base pair segment of the DNA polymerase gene was used. Their sequences are as follows: 5'-CATCACGACCCCGAGGGAG and 5'-GCGCCAGCCTGTTGTGTGTA. These are regions of identity in the genomes of HSV types 1 and 2 [6], and thus do not discriminate between these virus types. The reaction buffers and
conditions used were similar to published procedures [2,3], except that cycles were performed manually, with 90 sec each at 50°C, 65°C, and 94°C, for annealing, elongation, and denaturation, respectively. After 40 cycles of amplification, the reaction mixture was electrophoresed on a 5% NuSieve agarose gel (FMC Chemicals), and stained with ethidium bromide for visualization of DNA.

For control reactions, a pair of primers specific for the human beta-globin gene was used, as previously described [3].

RESULTS

To confirm the specificity of the HSV primers, we performed PCR on DNA from lungs known to be infected with HSV-1 or -2 by culture. Both samples gave the expected 92 base pair band after amplification, while DNA samples known to contain varicella-zoster virus, Epstein-Barr virus, or cytomegalovirus were not amplified (data not shown). These latter samples were obtained from autopsy livers with positive cultures, or cultured Raji cells, which is a Burkitt's lymphoma line with multiple copies of Epstein-Barr viral DNA. DNA was then purified from 11 blocks of skin biopsies. All samples proved to be of suitable quality for amplification, because the beta-globin gene was amplified with PCR (data not shown). PCR for HSV was then performed on those specimens. As can be seen in Fig 1a and Table I, all of the specimens with unambiguous histologic evidence of HSV gave an amplified band, while three normal skin controls did not. A single punch biopsy of a varicella lesion also gave no amplified band, confirming the lack of cross-reactivity of our assay for the varicella-zoster virus. In addition, three punch skin biopsies that showed histologic changes suggestive but not diagnostic of HSV, and which had clinical history compatible with HSV infection, also gave an amplified band. Therefore, this assay system is clearly superior to conventional histopathology.

Because the DNA extraction procedure used in the above experiments takes at least 24 h, we also performed PCR on DNA obtained by directly boiling deparaffinized tissue in buffer [5], as described in Materials and Methods. As can be seen in Fig 1b, a punch skin biopsy with known HSV infection gave a clear amplified band, while a control of normal skin was negative. By this method the entire assay can thus be finished in under 6 h.

DISCUSSION

We have shown in this paper that PCR offers a rapid, simple, sensitive, and specific test for HSV infections of skin and mucosa. This method can be performed on crude DNA prepared from minute piece (< 4 x 2 x 0.1 mm) of fixed and embedded tissues after cutting of sections for routine hematoxylin and eosin staining, and does not involve the use of radioisotopes. It is rapid, taking less than 1 h for sample preparation, less than 4 h for 40 cycles of reaction, and 1 h for gel electrophoresis. Furthermore, machines are now available that can perform the temperature cycling automatically, thus reducing technician time to approximately 1 h per run, which can include as many as 49 samples. All reagents needed are commercially available, and no complicated maneuvers or detailed knowledge of molecular biology is involved once the appropriate primers and optimal reaction conditions have been found and published. We therefore believe that this is a method suitable for routine clinical use.

REFERENCES