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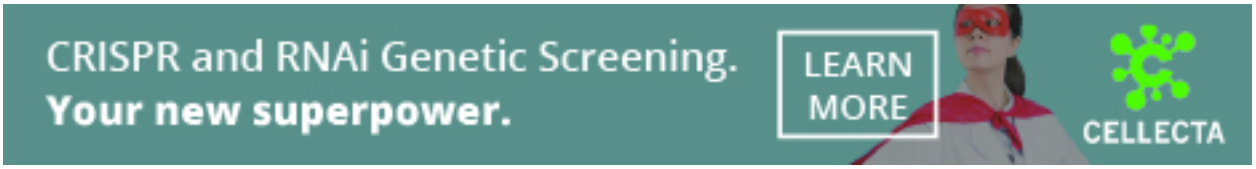

Detection of the MAIDS Virus Using the Polymerase Chain Reaction

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Murine AIDS (MAIDS) is an immunodeficiency syndrome that, like the human counterpart, is characterized by lymphadenopathy, hypergammaglobulinemia (IgM, IgG2a,IgG3), and profound T-cell dysfunction.[1–3] The etiologic agent of MAIDS has been identified as a defective retrovirus that primarily infects B lymphocytes.[4–6] This defective virus has been found to be able to induce MAIDS in susceptible strains of mice in the presumed absence of virus replication.[7,8] Upon cloning and sequencing of the virus the following were found: (1) a *gag* coding region capable of coding for a p15, p12, p30, and p10 (Fig. 1); (2) the p15, p30, and p10 sequences are highly homologous (81–95%) with the AKR murine leukemia virus (MLV), whereas the carboxyl end of the p12 coding region is only weakly homologous (30–40%) to MLV *gag* coding regions; (3) the virus is largely void of any *pol* and *env* coding sequences; and (4) the long terminal repeat (LTR) sequences are homologous to those found in ecotropic MLV endogenous to C57Bl/6 mice.[4,9]

Because a portion of the *gag* p12 sequence is the only region unique to the defective virus, many investigators have used it as a genetic probe to identify viral DNA and RNA in the cells of infected animals.[4,8,9] In previous investigations, these researchers have utilized the methods of Southern and Northern blot hybridization to identify virus DNA and RNA, respectively. We now describe the design and utilization of a set of primers that when employed in the PCR will allow for a more sensitive and specific detection of nucleic acid derived from the defective retrovirus responsible for MAIDS.

MATERIALS AND METHODS

Cell Lines and Virus

The following cell lines were used to assess the specificity of the PCR primers: (1) pAKV623, NIH 3T3 cells infected with an ecotropic murine leukemia virus (MLV); (2) NFS-Th-1-Xeno, a mink lung cell infected with a xenotropic MLV; (3) AKR247, a mink lung cell infected with a mink cell focus-forming virus (MCF); (4) MM5MT, a mouse mammary tumor cell line infected with the mouse mammary tumor virus (MMTV) obtained from the American Type Culture Collection; and (5) a mouse tail cell line derived from *Mus dunni*[10] that is permissive for MLV infection (kindly provided by Dr. S.K. Chattopadhyay, Laboratory of Cellular Oncology, National Cancer Institute). The MAIDS-producing virus mixture, LP-BM5, was kindly provided by Dr. J. Hartley (Viral Oncology Section, Laboratory of Immunopathology, National Institute of Allergy and Infectious Disease) and used to infect susceptible mice and the *M. dunni* cell line.

Isolation of Spleen DNA and RNA

Six-week-old female C57Bl/6 mice (Jackson Labs) were injected i.p. with 0.2 ml of LP-BM5 virus mixture. Two weeks after infection, their spleens were removed and processed for DNA and RNA. Spleens were removed from infected or noninfected mice, weighed, and placed in a plastic bag containing 1 ml of complete tissue culture media (RPMI-1640 + 5% fetal calf serum). To prepare a single-cell suspension from the intact spleen, the bag was placed in a Stomacher Lab Blender (Model 80, Seward Medical) and the spleen was homogenized using the HIGH setting for 2 min. The resulting single cell suspension was mixed with 9 ml of complete media, and then the cells were counted and pelleted. The cells were washed with 10 ml of phosphate-buffered saline (PBS), repelleted, and then suspended in 0.5 ml of PBS. An aliquot of the cells (0.25 ml) was used for DNA isolation and an aliquot (0.25 ml) was used for RNA isolation. The DNA was isolated using Proteinase K/SDS digestion and phenol extraction. Spleen cell RNA was isolated using the method described by Chomczynski and Sacchi.[11]

Conversion of Spleen cell RNA to cDNA

Spleen cell RNA (5 μg total cell RNA) from the cell lines or infected and noninfected mice was converted to single-stranded cDNA using the Cycle Kit purchased from InVitrogen (San Diego, California). The manufacturer's instructions were followed and yielded approximately 200 ng of single-stranded cDNA per reaction. One-tenth of cDNA was used as template for the PCR.

PCR

The oligonucleotide primers, p12-01 and p12-02 (Table 1), were synthesized using

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FIGURE 1 Structure of the MAIDS defective virus. A schematic of the defective virus is shown along with the location of the *gag*, *pol*, and *env* coding regions. Within the *gag* region are the coding regions for the p15, p12, p30, and p10 (dark rectangles). The region containing the sequences unique to the defective virus is represented by a white box within the p12 coding region. The arrowheads depict the location of PCR primers.

the Applied Biosystem's 392 DNA/RNA synthesizer (Foster City, California). The 3′ primer (p12-02) of the p12 pair is contained within the sequences unique to the defective virus while the 5′ primer (p12-01) is contained within the p15 coding region that is not unique to the virus. However, due to the uniqueness of the 3′ primer, the p12 primer pair will amplify a 209-bp fragment that is specific for the MAIDS defective virus.

Spleen cell DNA (1 μg) or cDNA (approximately 20 ng) from infected or noninfected mice was added to a 100-μl PCR reaction mix containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, and 600 ng of each primer. The reaction mixture (minus the *Taq* polymerase) was overlaid with mineral oil, heated to 95°C for 5 min and cooled to 80°C, and then 2.5 units of *Taq* polymerase were added. The PCR reaction was performed for 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The amplified DNA was analyzed by electrophoresing 10 μl of the reaction mixture in a 2% agarose gel containing 0.5 μg/ml of ethidium bromide and was then visualized with a UV light source.

Sequencing of PCR Products

The PCR products obtained from amplification of the infected cell DNA were purified using the Magic PCR Preps DNA Purification System (Promega, Madison, Wisconsin). The purified DNA (5 μl) was then sequenced using the fMole kit purchased from Promega. The PCR primers (50 ng each) were end-labeled with [γ-^{32}P]ATP and used to prime the sequence reaction. Sequencing was performed according to the instructions provided with the fMole kit. The products of the sequence reaction were separated on a 5% Hydrolink/7 M urea gel using constant power (70 watts) and then exposed to Kodak XRP film for 3–12 hr. The resulting sequences were analyzed using the Intelligenetics Suite software (Mountain View, California).

RESULTS

To determine specificity of the PCR primers, infected cell DNA was obtained from cell lines infected with ecotropic or xenotropic MLV, with MCF, or with MMTV and used as template for the PCR. As shown in Figure 2a, the MAIDS p12-specific primers only amplified the target from cells infected with the MAIDS virus (lane 3). When *M. dunni* cells or splenocytes obtained from C57Bl/6 were used as the source of cell DNA, no PCR products were obtained (data not shown). The sensitivity of the PCR was assessed by mixing MAIDS infected cells with noninfected *M. dunni* cells. As shown in Figure 2b, we could really detect as few as 5 infected cells per 10^6 cells (lane 9) by visualization of the ethidium bromide-stained agarose gel under ultraviolet light.

When the PCR was performed using DNA or RNA from the splenocytes of infected mice it was observed that the primer pair produced a product of the predicted size (Fig. 3, lane 4, and Fig. 2, lane 6, respectively). The DNA and RNA obtained from the spleens of noninfected mice failed to produce PCR products (Fig. 3, lanes 3 and 5, respectively). These results again suggest that the primer pair is specific for the MAIDS virus and can be used to detect the presence of virus in cell culture and in infected animals.

To verify that the PCR products produced were indeed derived from the defective virus, the PCR product amplified from the DNA of infected mice was sequenced. The sequence obtained from the PCR product was homologous to that contained in the GenBank submission M64096 and therefore indicated that the primer pair is indeed specific for the defective MAIDS virus (data not shown).

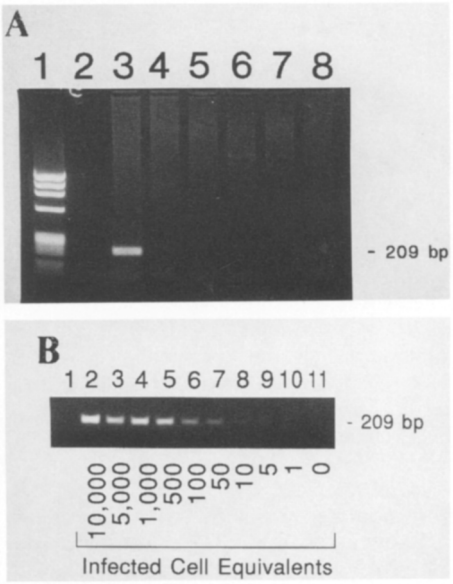

FIGURE 2 Determination of the specificity and sensitivity of the MAIDS-specific PCR. (*A*) The specificity of the PCR is demonstrated. (Lane *1*) Molecular weight marker DNA (*Hae*II-digested ϕX 174); (lane *2*) reagent control; (lane *3*) LP-BM5-infected spleen cell DNA as template; (lane *4*) noninfected spleen cell DNA; (lane *5*) ecotropic MLV-infected cell DNA; (lane *6*) xenotropic MLV-infected cell DNA; (lane *7*) MCF-infected cell DNA; (lane *8*) MMTV-infected cell DNA as template for the PCR. (*B*) The sensitivity of the MAIDS-specific PCR is demonstrated. (Lane *1*) reagent control; (lanes *2–11*) template is 1 μg of DNA obtained from a mixture of 10^6 cells containing 10,000, 5000, 1000, 500, 100, 50, 10, 5, 1, and 0 infected cells, respectively. The size of the PCR products is shown as base pairs (bp).

TABLE 1 PCR Primer Sequences

Primer	Sequence	Length (nucleotides)	Position[a]
p12-01	CCTTTATCGACACTTCCCTT	20	1288
p12-02	CCGCCTCTTCTTAACTGGTC	20	1497

[a]The position is derived from the GenBank submission, M64096.

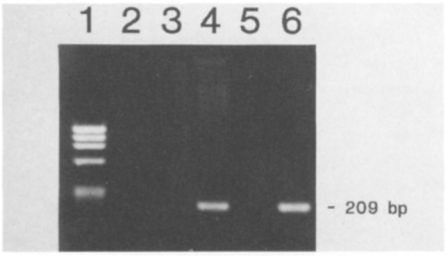

FIGURE 3 Analysis of the defective virus-specific PCR. The results of the defective virus-specific PCR are shown in this ethidium bromide-stained agarose gel. (Lane *1*) Marker DNA (ϕX 174 *Hae*III fragments); (lane *2*) reagent controls (PCR mix with no template DNA); (lane *3*) noninfected spleen cell DNA as template; (lane *4*) LP-BM5-infected spleen cell DNA as template; (lane *5*) cDNA prepared from noninfected spleen cell RNA; (lane *6*) cDNA prepared from LP-BM5-infected spleen cell RNA as template. The sizes of the PCR products are shown in base pairs (bp).

DISCUSSION

The investigation of the human immunodeficiency virus type 1 (HIV-1) and its interaction with the immune system has certainly been bolstered by the availability of the PCR. The PCR is being used not only to assist in the diagnosis of HIV-1 infection but also to assist in the search for compounds and vaccines that will prevent, protect against, or retard HIV-1 replication.

To investigate the interactions of the MAIDS-defective retrovirus with the murine immune system, many investigators have relied on immunofluorescence staining and Southern and Northern blot hybridization. However, since the use of genetic probes encompassing the unique regions of the MAIDS virus has been questioned (due to potential cross-reactivity with other viruses) and does not provide the sensitivity needed when working with a small number of infected cells, we sought to produce defective virus-specific primers that could be used in the PCR. By exploiting the unique features of the MAIDS-defective virus, we have produced a primer pair that allows for the rapid and specific detection of the virus. As demonstrated, these primers can be utilized to detect the presence of both proviral DNA and viral RNA early in the development of MAIDS. To understand further the process by which MAIDS is induced, we are presently using these primers to analyze the cellular tropism and the kinetics of viral gene expression in infected mice. The sensitivity of the PCR and the specificity of these primers will permit a more detailed analysis of the mechanisms associated with defective-virus gene expression and the development of the murine immunodeficiency syndrome.

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