GENETICS

Transfer of Genetic Information of Type D Retroviruses from Lymphocytes of Patients with B-Cell Lymphosarcoma to Raji Cells

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Polymerase chain reaction and immunoblotting show that the sequences homologous to the *pro-pol* domain of Mason—Pfiser monkey virus can be transferred from lymphocytes of patients with B-cell lymphosarcoma to Raji cells in a cell culture.

Key Words: *type D retroviruses; B-cell lymphosarcoma*

Exogenous type D retroviruses are lymphotropic viruses isolated from some monkey species and containing no oncogenes. Antibodies to these viruses were detected in the healthy population of West Africa [5] and in patients with lymphadenopathies, including AIDS [4]; type D retrovirus was isolated from a patient with B-cell lymphoma and AIDS [2].

Detection of nucleotide sequences in the lymphocytes of children with B-cell lymphosarcoma [3] prompted us to study the transfer of provirus sequences from patient's lymphocytes to a stable cell line.

MATERIALS AND METHODS

Raji human B-cell line and Jurkatt human T-cell line permissive for type D retroviruses were used. Lymphocytes of patients with B-cell lymphosarcomas were isolated from peripheral blood conserved with glugicir by centrifugation on a Ficoll-containing medium for lymphocyte isolation (Flow Lab). The lymphocytes were cultured in RPMI with 20% fetal serum and antibiotics. Once active proliferation of lymphocytes started, Raji or Jurkatt cells were added and then cultured for 4 to 8 months. DNA from patients' lymphocytes and from lymphocytes of mixed cultures was isolated using proteinase K and phenol-chloroform extraction [1].

The sequences homologous to the *pro-pol* domain of Mason—Pfiser monkey virus (MPMV) were detected by the polymerase chain reaction (PCR) [6] using primers 5'-AGG-GGC-CAG-CCC-CAG-GCC-CC-3' (position 2720 to 2739) and 5'-TGG-GGT-GCA-AGT-ATG-TCA-ATG-GVV-C-3' (position 3652 to 3627). The PCR was conducted in 30 cycles, the parameters of each cycle being as follows: 96°C, 30 sec; 55°C, 1 min; 72°C, 2 min. The specificity of DNA fragments amplified by PCR was assessed by hybridization with plasmid containing an insertion of the full-length MPMV genome (a generous gift of Dr. E. Hunter, USA). Construction of the plasmid and hybridization were performed following DIG DNA protocol, Labeling and Detection Kit (Boehringer).

Sera of patients with B-cell lymphosarcomas diluted 1:100 were analyzed for the presence of antibodies to type D retrovirus by Western blot [7]. Mason—Pfiser monkey virus and type D virus from

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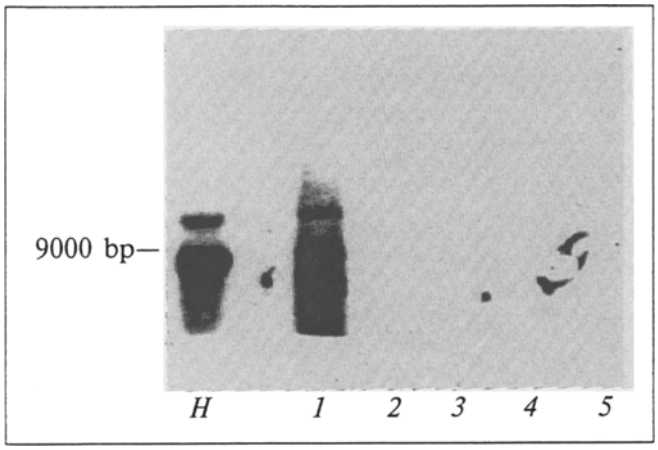

Fig. 1. Detection of specific sequences of type D retroviruses in cultures containing lymphocytes of patients with B-cell lymphosarcomas and Raji cells by polymerase chain reaction using primers homologous to *pro-pol* domain of the Mason—Pfiser virus genome and specific hybridization with digoxigenin-labeled probe. *H*) DNA from HEp-2 cells; *1*, *2*, *3*, *5*) DNA from cell cultures containing patients' lymphocytes and Raji cells; *4*) DNA from intact Raji cells.

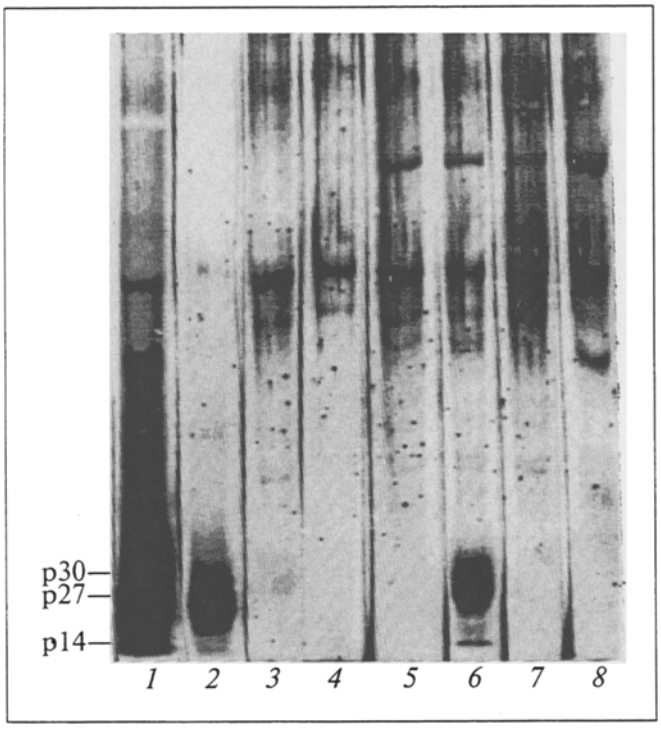

Fig. 2. Detection of structural proteins of type D retrovirus in the supernatant of culture containing lymphocytes from a patient with B-cell lymphosarcoma and Raji cells by Western blot with antiserum to *gag*-coded proteins of Mason—Pfiser virus (MPMV). Supernatant of HEp-2 cells (*1*), MPMV (*2*), Raji (*3*), Jurkatt (*4*); supernatants of cultures containing patients' lymphocytes and Raji cells (*5* and *6*) or patients' lymphocytes and Jurkatt cells (*7* and *8*).

HEp-2 cell line (HEp-2V) were purified on a sucrose gradient and used as antigens.

Supernatants of cultures containing patients' lymphocytes and Raji or Jurkatt cells and the supernatants of Raji and Jurkatt cell cultures (control) were studied as follows: the cultures were ultracentrifuged, the pellet was fractionated on 20-60% sucrose gradient, and the fraction floating at d=1.16 g/ml was analyzed by Western blot for the presence of proteins similar to structural proteins of MPMV. Antiserum to *gag*-coded MPMV proteins was used.

RESULTS

Fourteen cultures containing lymphocytes of patients with B-cell lymphosarcomas (2-14 years) and of Raji or Jurkatt cells were prepared. Two cultures were of special interest. One of them contained Raji cells and lymphocytes isolated from peripheral blood of a 13-year-old boy with B-cell abdominal lymphosarcoma. The patient's serum reacted with the major MPMV structural protein p27 in Western blot. Sequences homologous to the *pro-pol* domain of the MPMV genome were revealed in lymphocytic DNA by PCR with subsequent hybridization. After simultaneous culturing of these lymphocytes and Raji cells, sequences homologous to *pro-pol* domain of the MPMV genome were present in the DNA isolated from these cultures. In Fig. 1, hybridization spots are seen in track *1* (DNA from culture containing patients' lymphocytes and Raji cells) and in track *H* (HEp-2 DNA), but not in tracks *2*, *3*, and *5* (DNA from cultures containing patients' lymphocytes) and track *4*, (DNA from Raji cells). Specific proteins reacting with antiserum to *gag*-coded MPMV proteins were not detected in the culture medium.

The other culture contained Raji cells and peripheral blood lymphocytes from a 7-year-old boy with the same sarcoma. Serum anti-MPMV p27 antibodies were revealed by Western blot. PCR with subsequent hybridization showed that DNA from peripheral blood lymphocytes and mixed cultures contains sequences homologous to *pro-pol* domain of the MPMV genome. Western blot revealed 30 and 14 kD proteins in the protein fraction (d=1.16 g/ml) obtained by sucrose gradient ultracentrifugation of cultured cells. These proteins reacted with antiserum to *gag*-coded MPMV proteins (track *6*, Fig. 2). These proteins were not found in the culture media from Raji and Jurkatt cells (tracks *3* and *4*, Fig. 2) and from three other cultures containing patients' lymphocytes (tracks *5*, *7*, and *8*, Fig. 2).

Thus, genetic information of type D retrovirus can be transferred from patients' peripheral blood lymphocytes to Raji cells during simultaneous culturing. It is obvious that several months in such a culture minimizes the persistence of patient's lymphocytes, therefore, genetic information of type D retrovirus is carried by Raji cells. It is noteworthy that the *pro-pol* sequences were detected in two cultures,

whereas *gag*-coded proteins were expressed only in one. Moreover, if the *pro-pol* sequences were detected in other cell culture, the amount of 30 and 14 kD proteins reacting with serum against *gag*-coded proteins of MPMV varied from culture to culture. An important result of this study is a convenient cell culture model for investigation of genetic information of type D retroviruses from the lymphocytes of patients with B-cell lymphosarcomas.

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The Role of Genetic Differences in the Mother—Fetus System in Biotransformation of Proteratogens

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Culturing of postimplantation embryos of C57Bl/6 and DBA mice differing in the ability to biotransform 3,4-benz(a)pyrene, reciprocal crossing of these animals, and transfer of preimplantation embryos into sham-pregnant females is used to model different genetic states by the Ah locus in the mother—fetus system. The role of metabolic factors at the early stages of embryogenesis in the realization of embryo- and genotoxic effects of 3,4-benz(a)pyrene is evaluated.

Key Words: *embryonal metabolism of xenobiotics; teratogenesis; sister chromatid exchanges*

A variety of synthetic compounds are biotransformed in human and animal body with formation of teratogenic, mutagenic, and/or blastomogenic metabolites. Deleterious effects of such a transformation on rat embryos at the initial stages of organogenesis have been demonstrated for cyclophosphamide, ethanol, adriamycin, and other compounds [2,3,5]. Recent studies show that metabolic activation of some substances, specifically, polycyclic aromatic hydrocarbons (PAH), aromatic amines, and dioxines occurs not only in mother's body, but also in embryos at the early stages of prenatal life [6,7]. The ability to transform PAH is controlled by the Ah locus and is inherited by the autosomal monogenic pattern as a dominant trait [8]. Some animals, for example, C57Bl/6, CBA, and C3H mice, carry the dominant allele Ah^b and are homozygous for this allele (Ah^b/Ah^b), indicating a high ability to biotransform PAC. Some other mouse strains (DBA, AKR, and SWV) are homozygous for the recessive allele (Ah^d/Ah^d) and exhibit a weak response to PAH [8].

The discovery of genetically determined ability to bioactivate xenobiotics in adult animals and early embryos reshapes traditional concepts concerning the teratogenesis of chemicals in mammals. The contribution of metabolic factors to this process at the early stages of embryogenesis is unclear, particularly in cases when mother but not fetus carries the recessive alleles Ah^d. This problem is particularly im-

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