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Invited Paper

PCR Technique in the Study of Malignant Lymphoma

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Since the unveiling of the polymerase chain reaction (PCR) method of DNA amplification at the American Society of Human Genetics Conference in 1985, numerous modifications, improvements and novel applications of PCR have been developed. In this study, we introduce the PCR usage of our department. The targets of the PCR were viruses, translocations and rearrangements of DNA, as well as the expression of RNA and DNA on the sections.

HTLV-I Associated Lymphadenitis[1)]

We examined the histopathologic changes of the lymphnodes from 10 patients with mild lymphadenopathy, a few atypical lymphocytes in the peripheral blood, skin lesions, and proviral DNA of human T-cell leukemia virus type I (HTLV-I) in the nodes. The proviral DNA of HTLV-I was detected by a Southern blot analysis, the in situ hybridization technique, and/or PCR. The lymph nodes showed preserved nodal architecture with a diffuse infiltration of small to intermediate sized lymphocytes in association with scattered transformed lymphocytes and a few immunoblast-like cells in the enlarged paracortex. Eight of 10 patients received no therapy, and all patients were still alive and healthy more than 5 months after the biopsies. The histology resembled than of a viral infection and could be clearly distinguished from HTLV-I associated lymphomas. The PCR findings showed that the presence of the HTLV-I provirus has no association with either neoplastic or reactive states.

Defective provirus form of human T-cell leukemia virus type I in adult T-cell luekemia/lymphoma: clinicopathologic features[2)]

Human T-cell luekemia virus type I (HTLV-I) is associated with adult T-Cell luekemia/lymphoma (ATLL). To examine the relationship between defective HTLV-I proviruses and clinicopathologic features, we examined 95 patients with ATLL showing a clonal integration of HTLV-I proviral DNA; 77 patients (81%) showed one clonal band, 15 (16%) showed two clonal bands, and 3 (3%) showed three clonal bands. In addition, a defective proviral from was detected in 28 patients (29%): 23 (30%) of the 77 with one clonal band, 4 (27%) of the 15 with two clonal bands, and 1 (33%) of the 3 with three clonal bands. The number of clonal bands demonstrated no association with the presence of defective proviruses. We classified the 95 patients with ATLL into four types accoding to their clinicopathologic features (smouldering leukemia, chronic leukemia, acute leukemia, and lymphoma types). The distribution of patients with the defective form did not differ among these four types. All patients with the defective form showed a defect in the gag or/and env region, while no patient had any defect in the pX region. Thus false negative results would likely be obtained, if we had chosen the defective region as the primer site.

Bcl-2 gene and prognosis of B-cell lymphoma[3)]

Both the PCR and a Southern blot analysis were used to determine the bcl-2 gene rearragement in B-cell lymphoma. Recent molecular studies have shown that the translocation of karyotypic abnormalities t(14; 18) result in the juxtaposition of the candidate proto-oncogene bcl-2 on chromosome 18 with the immunoglobulin heavy-chain locus on chromosome 14. We detected the bcl-2 rearrangement in three of six follicular lymphomas (50%) , two of five follicular and diffuse lymphomas (40%), one of 13 diffuse medium-sized cell lymphomas (7.7%) and two of 33 diffuse large cell lymphomas (6.0%) through a Southern blot analysis. With PCR, the rearrangement was demonstated in five of eight follicular (63%), three of five follicular and diffuse (60%) , seven of 36 diffuse large cell lymphomas (19%) and two of 13 diffuse medium-sized lymphomas (15%). Diffuse large cell lymphoma with a bcl-2 rearrangement in PCR represented a good prognosis as well as follicular lymphoma. The bcl-2 gene was closely related to follicular lymphoma as in previous reports and demonstrated the general prognostic importance in diffuse large cell lymphoma of B cell type.

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Amplified bcl-2/JH rearrangements in reactive lymphadenopathy[4]

The PCR was performed, to examine the ability of the joined bcl-2/JH in the process of the mistaken t(14;18) of the reactive lymphoadenopathy. We detected amplified DNA in two of 18 lymph nodes. The PCR of the two nodes were thus performed again. But we detected no amplification at the second time. So the amplified DNA was considered, to orginate from one copy of the joined bcl-2/JH of one cell. These results suggest that the mistaken joined bcl-2/JH at the juncture of the t(14; 18) had thus occured in the reactive lymph nodes. We threrfore attempted to carefully evaluate the meaning of these PCR products.

Monoclonal B Cells and Restricted Oligoclonal T cells in T-cell-Rich B-Cell Lymphoma[5]

Immunophenotyping of lymphoma using paraffinembedded lymphoid tissue is useful in identifying the large neoplastic B cells in T-cell-rich B-cell lymphoma (TRBL), but does not succeed in deciding clonality. We studied six cases, to determine the clonal population of B and T cells of TRBL. Immunohistochemistry on frozen and paraffinembedded material showed that the cellular population in all six cases consisted mainly of T cells; while fewer than ten percent of the cells stained as B cells. However, in all cases, monoclonality of the immunoglobulin was helpful in diagnosing the B-cell neoplasia. A Southern blot yielded genetic analysis showed the monoclonality of B cells in three cases, but no evidence of clonality in the T cells. In addition, gene monoclonality has also been detected in all cases examined by PCR, using the primers for the V and J regions of the immunoglobulin heavy chain gene. For T cells, the D and J regions of the T-cell-receptor (TCR) β chain showed the same patterns of the oligoclonal bands in all cells, while the V and J regions of the TCR γ chain showed the same bands in all. The expression of the TCR V β families was polyclonal but restricted.

Clonal Analysis of Hodgkin's Disease[6]

To better characterize the clonality and pathogenesis of Hodgkin's disease (HD), we used both the PCR and a Southern blot analysis to analyze the rearrangement of immunoglobulin and T-cell receptor genes (TCR), and the bcl-2 oncogene. Twenty-six cases of HD were compared with 15 cases of nonspecific lymphadenitis, and 7 of incipient adult T-cell leukemia/lymphoma (ATLL), and 4 of T-cell rich B-cell lymphoma (TRBL), all of which histologically resembled HD. All TRBLs showed the rerrangement of immunoglobulin genes by PCR and/or Southern blot, and all incipient ATLLs displayed the rearrangement of the TCR genes. In HD, however, PCR and Southern blot displayed only two cases of TCR gene rearrangement, while two others had a very weak rearrangement of the immunoglobulin gene based only on the PCR findings. Only one of 26 cases of HD showed bcl-2 gene rearrangement by PCR, while no other disease did. We were not able to detect a clonal population in most cases of Hodgkin's disease.

Genetic changes in atypical hyperplasia and lymphoma with angioimmunoblastic lymphadenopathy and dysproteinaemia in the same patients[7]

The transition between atypical hyperplasia and lymphoma with angioimmunoblastic lymphadenopathy and dysproteinaemia (AILD) was studied in serial lymph node biopsies. Specimens from five patients using a DNA analysis with a Southern blot analysis and PCR. The chromosomal analysis showed additional abnormalities as the disease progressed in comparison to those initially present. In the first biopsy from each patient a diagnosis of atypical hyperplasia with AILD was made and lymphoma was excluded due to the finding of only a few atypical lymphoid cells and the preservation of the follicles with germinal centers. A DNA analysis of lymph nodes at this stage showed either germ lines or oligoclonal rearrangements of the T-cell receptor (TCR) and immunoglobulin heavy chain genes. In the final biopsy, when a diagnosis of lymphoma with AILD was made, either a monoclonal rearrangement of the TCR was observed or one of the rearranged bands had increased in density. These results suggest that a selective proliferation of a clone of abnormal cells may account for the progression of atypical hyperplasis to lymphoma with AILD.

Analysis of Epstein-Barr Viral Genomes in Lymphoid Malignancy Using Southern Blotting, and PCR[8]

109 cases of malignant lymphoma were surveyed by a Southern blot analysis and polymerase chain reaction (PCR) for Epstein-Barr virus (EBV) DNA while 16 cases of non neoplastic lympeadenopathy and 4 of normal thymus were compared. In positive cases determined by Southern blot and PCR methods. In the Southern blot analysis, two of seven Hodgkin's disease samples (29%) (one each of mixed cellularity and lymphocyte predominance), three of 56 B cell lymphomas (5.6%) and five of 46 T cell lymphomas (11%) demonstrated EBV DNA. However, 16 cases of lymphadenitis and 4 with a normal thymus showed no EBV DNA. With PCR, one case of B cell lymphoma, nine of T cell lymphoma, ten of lymphadenitis and two of the thymus represented the DNA in addition to positive cases determined by the Southern blotting method. These results indicate that the presence of EBV DNA is not related to lymphoid malignancy, but an enhancement of the DNA is demonstrated in some neoplastic conditions.

Heterogeneity of EBV Infection in AILD type T-cell Lymphoma[9]

To investigate the relationship of EBV and angioimmunoblastic lymphadenopathy with dysproteinemia (AILD), we performed a DNA analysis using the PCR,

Southern blot, in situ hybridization, and an immunohistochemical analysis of the lymph nodes in five patients who were followed up and biopsied more than once. The EBV nucleic acid sequences were found by either PCR or in situ hybridization in all examined nodes. The number of EBV-positive cells varied widely among the cases and throughout the course of the disease in the same patients. The analysis of EBV terminal repeats (TRs) or lymphocyte-determined membrand antigen genes showed polyclonal populations in the EB-infected cells. EBV-positive cells possessed intermediate to large-sized nuclei, and the cells with large nuclei, were observed to particularly express the latent membrane protein of EBV. These large cells varied in number among the cases. Double-labeling immunohistochemistry/in situ hybridization studies demonstrated that most of the EBV-positive cells expressed B-cell antigen (CD20). The EBV presence thus seems to be associated with the selective defects of the immune system, rather than with the direct pathogenesis of AILD.

Demonstration of EBV, Using PCR-ISH[10)]

We used the polymerase chain reaction (PCR) in situ hybridization (ISH) (PCR-ISH) on sections of malignant lymphoma and nonspecific lymphadenitis to detect small amounts of EBV, a DNA virus of the herpesvirus family. We first surveyed the EBV DNA by a Southern blot analysis and PCR, and then compared the results of the two PCR/ISH methodologies with the results of simplified/sensitive ISH for the positive cases. The target of the simplified in situ (DNA-ISH) was a few copies of EBV DNA per cell, while the target of the sensitive in situ (RNA-ISH) was as many as 10^7 copies of EBV RNA per cell. When EBV DNA was detected by Southern blot, DNA-ISH, RNA-ISH and PCR-ISH all revealed EBV genomes. When PCR revealed only amplified EBV DNA, DNA-ISH showed no EBV genomes, but PCR-ISH and RNA-ISH showed EBV genomes in a few cells. When PCR showed no detectable amplified EBV DNA, all of DNA-ISH, RNA-ISH and PCR-ISH showed no genomes. These findings indicate that PCR-ISH consistently detected a few copies of the EBV virions. The PCR-ISH was as sensitive as RNA-ISH. The RNA-ISH could not detect a virus if RNA was not expressed, but the PCR-ISH could detect a virus without such expression.

Finally, these PCR methods are considered to be both useful and sensitive. However, extreme care must be taken to distinguish whether the PCR products indicate a direct or indirect pathogenesis.

Key words: PCR, malignant lymphoma

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