Clinical Science Workshop 6

Viral opportunistic infections

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99 Evaluation of a JC virus-specific immunoglobulin M enzyme linked immunosorbent assay

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Objectives: Progressive multifocal leukoencephalopathy (PML) is caused by a reactivated infection with the human polyomavirus JC (JCV). Primary infection with JCV has not been linked to any disease in humans. Using recombinant viral protein 1 (VP1) of JCV as antigen, we developed an enzyme linked immunosorbent assay (ELISA) for the detection of VP1-specific IgM.

Methods: Two different direct ELISA methods, two capture ELISA methods and an indirect ELISA were evaluated. Only the indirect ELISA yielded reproducible results. As standard ten sera with known titers of IgM antibodies to JCV were used in each assay. Human sera negative for IgG antibodies to VP1 were used as negative controls.

Results: Evaluating 24 JCV-IgG antibody negative human sera the cut-off was identified at 5× mean optical densities (OD). All sera with a reproducible OD above 563 mOD were defined as positive. Of the ten reference sera with known titers, three were reproducibly positive by ELISA. The serum 81/11455 yielding the highest OD was used as internal reference. In a cohort selected for age, i.e. with groups of 20 for each age group from 0–10 to 90–100 years, four of 363 sera (1.04%) were positive. Of these patients two suffered from infections of the genitourinary tract, one was pregnant and one was treated with systemic corticosteroids.

Conclusion: Using recombinant VP1 in an indirect ELISA, human IgM antibodies to JCV-VP1 can be reliably measured. Interference with specific IgG, rheumatoid factor or instability of IgM molecules appear not to be relevant in this test. Urinary tract infections, pregnancy and immunosuppression are also known to cause BKV infections. Given the known amino acid similarities between JCV-VP1 and BKV-VP1, crossreactions between these viruses may account for these positive results. As recombinant VP1 of BKV was not available for evaluation, this could not be determined at this time.

100 Detection of cytotoxic T lymphocytes specific for an HLA A*0201-restricted epitope of JC virus VP1 protein in healthy individuals

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Introduction: In a previous study, using a computer predictive analysis, we have found an HLA A*0201-restricted cytotoxic T lymphocytes (CTL) epitope on the VP1 protein of JC virus (JCV)(1). The specificity of the computer algorithm, which predicts CTL epitopes solely on the basis of the expected binding affinity of nonamer peptides to the HLA A*0201 molecule, proved to be quite limited: only 1 of 11 predicted nonamer peptides turned out to be a valid JCV CTL epitope.

Methods: We performed an epitope mapping study of the VP1 protein using overlapping peptides. A total of 29 20-amino acids (aa) peptides (20-mers) overlapping by 8 aa and spanning the entire JCV VP1 protein were synthesized.

Results: Using a 51Cr release assay, we found a 20-mer located close to the N-terminal region of the VP1 protein, which was recognized by the CTL of HLA A*0201 HIV+/PML survivors. A fine mapping of this 20-mer, revealed the presence of a 9-mer epitope, p2, which elicited a strong CTL response. We then constructed an HLA A*0201/JCV VP1p2 tetrameric complex. Using this technology, we were able to assess the presence of JCV VP1p2-specific CTL in a total of 26 subjects including different categories of patients along with healthy controls. Thus far, we have found that VP1p2-specific CTL were detectable not only in 7/8 PML survivors, but also in 2/2 HIV+ patients with JCV-negative leukoencephalopathy resembling PML. 1/2 HIV+ patient with a neurological disease unrelated to PML, as well as in 4/4 healthy individuals. Vp1p2-specific CTL could not be detected in 7 PML progressors.

Conclusion: This is the first demonstration of JCV-specific CTL in healthy individuals. We hypothesize that, even in low number, these cells are sufficient to keep JCV under control and prevent the onset of PML. 1) Koralnik I.J., Du
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JCV genotypes and transcriptional control region analysis in long survivor and nonresponder PML patients under HAART treatment

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Although the introduction of highly active anti-retroviral therapy (HAART) has been reported to reduce the incidence of most AIDS-related opportunistic infections, the improvement of survival occurs only in about the 50% of progressive multifocal leukoencephalopathy (PML) cases. In addition to acquired immunodeficiency and host-related factors, it has been hypothesized that JCV particular genomic organizations could influence the course of PML as a result of changes in the viral fitness.

In order to evaluate this hypothesis, nucleotide sequence analysis was performed to characterise the JCV major capsid protein (VP1) genomic region, mostly involved in the virus/host cell interaction and the transcriptional control region (TCR). JCV DNA was amplified in CSF samples collected from 17 Italian AIDS patients (6 females, 11 males, mean age 38 years) with virologically confirmed PML. Following HAART, PML stabilised in 9 patients leading to survival times of >2 years (responders, R), while 8 patients died within six months from the onset (nonresponders, NR).

The study of VP1 region showed that JCV type 1, amplified in the CSF samples collected from 5 R and 5 NR patients, was the most represented genotype, followed by JCV type 2, that was found in 2 R and 2 NR patients, and genotype 4, recognised in 2 R and 1 NR patients.

The analysis of 9 amplified TCR regions showed in the CSF samples of both R and NR patients the exclusive presence of unique and extensive archetype-derived rearrangements (type II), with duplications of transcription factor binding sites, in particular the AP-1/c-jun binding motif. Surprisingly, we found in the CSF of 2 PML R patients a TCR organization almost identical to the archetypal form.

In conclusion, our preliminary results, don’t indicate the existence of specific JCV genomic markers for PML outcome prediction in course of HAART treatment. Further studies involving larger number of R and NR PML patients are needed to confirm these observations.

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In HIV+ patients with progressive multifocal leukoencephalopathy, anti-JC virus CD4 T-cell responses are recovered following prolonged HAART

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Background: JC virus (JCV) is the causative agent of progressive multifocal leukoencephalopathy (PML). JCV-specific CD8 T cell response has been shown to play a crucial role in the control of JCV infection. In several viral infection models, CD8 T cell response requires help from CD4 T cells. Here we examined anti-JCV specific CD4 T-cell response in several groups of healthy and HIV+ patients with or without PML.

Methods: Anti-JCV CD4 responses were investigated by means of a proliferation assay to purified JCV. Four groups of individuals without PML were defined: healthy subjects and HIV+ patients with or without positive JCV DNA detection by PCR in urine. Two further groups of HIV+ patients with PML were considered: i) patients at the onset of PML starting on HAART and ii) PML survivors on prolonged HAART (over at least 6 months).

Results: No significant anti-JCV CD4 T-cell proliferation was found neither in healthy subjects (n=5) nor in HIV+ patients (n=12) with no JCV excretion in urine. All healthy subjects (n=9) and 7 of the 13 HIV+ patients (mean CD4 = 254) with detectable JCV excretion in urine had a positive anti-JCV CD4 T cell response. No significant response was found in the 14 patients (mean CD4 = 100) with starting PML at the onset of HAART, while 8 of the 11 PML survivors (mean CD4 = 241) had a positive anti-JCV CD4 response following prolonged HAART. Of interest, the negativation of JCV PCR was observed in 10 out of 11 PML survivors. Considering all HIV+ patients including PML and non-PML groups, those with a positive anti-JCV CD4 response had a significantly higher CD4 cell count than non-responders (Mann Whitney U test, p < 0.01).

Conclusions: i) Anti-JCV CD4 T cell response was mostly detected in subjects with both active JCV infection and CD4 cell count over 100 cells/µL. ii) A lack of anti-JCV CD4 T cell response was constantly observed in patients at the onset of PML, however it was also observed in some HIV+ patients without PML. Therefore the occurrence of PML might require additional factors. iii) Prolonged HAART led to the restoration of anti-JCV CD4 T cell response in PML survivors and a negativation of JCV PCR in CSF.