Epstein-Barr virus (EBV) – A main cause of rheumatoid arthritis.

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Received December 28, 2016

Objective.
Many studies presented some evidence that EBV might play a role in the pathogenesis of rheumatoid arthritis. Still, there are conflicting reports concerning the existence of EBV in the synovial tissue of patients suffering from rheumatoid arthritis.

Material and methods.
Takeda et al. designed a study to detected EBV DNA is synovial tissues obtained at synovectomy or arthroplasty from 32 patients with rheumatoid arthritis (RA) and 30 control patients (no rheumatoid arthritis). In this study, the data as published by Takeda et al. were re-analysed.

Results.
EBV infection of human synovial tissues is a condition per quam of rheumatoid arthritis. And much more than this. There is a highly significant causal relationship between an EBV infection of human synovial tissues and rheumatoid arthritis ($k=+0.546993718$, p-value = 0.00001655).

Conclusion.
These findings suggest that EBV infection of human synovial tissues is a main cause of rheumatoid arthritis.

Introduction.
Rheumatoid arthritis (RA), a systemic, predominantly $^1$ CD4+ T helper type 1 (Th1)-driven disease characterized by an extensive synovial hyperplasia and infiltration by macrophages, monocytes, lymphocytes and fibroblasts, is a destructive, chronic and debilitating arthritis. RA affects more or less about 1% of the world’s population. The prevalence of rheumatoid arthritis in men is twofold less $^3,4$ than in women. The long-term prognosis of rheumatoid arthritis remains very poor. In particular, the average life expectancy is reduced by 3 to 18 years $^5$. The loss from the workplace, the indirect costs of disability and the direct costs of treatment of RA are very high $^6$. At present there is no known cure for rheumatoid arthritis. Many exposures investigated as possible risk factors for the development of rheumatoid arthritis such as dietary factors (antioxidants) $^8,9$, red meat protein $^{10,11}$, fat intake $^{12,13}$, breast feeding, the use of oral contraceptives or hormone replacement therapy $^{14,15,16}$ have shown no strong associations. Only cigarette smoking has been found to increase the risk of rheumatoid arthritis $^{17,18,19,20}$. In the quest to uncover the unknown etiology of rheumatoid arthritis, viruses including Epstein–Barr virus (EBV), human herpesvirus-6, human herpesvirus-8, parvovirus B19, HTLV-1, and human endogenous retroviruses-5 have all been hypothesized for many years to be involved in the pathogenesis of rheumatoid arthritis $^{21,22,23,24,25,26,27,28,29}$. Many studies presented some evidence suggesting that especially EBV might play a role in the pathogenesis of RA. Among them Alspaugh and Tan $^{30}$ were one of the first. However, due to conflicting reports concerning the existence of EBV in the synovial tissue of...
RA patients, a cause or the cause of rheumatoid arthritis, a highly disabling systemic autoimmune disease, remains unknown.

Material and methods

Study design
Takeda et al. designed a study to evaluate the presence of the EBV genome in the synovial tissue of RA patients and to localize the EBV-infected cells. Synovial tissues were obtained at the time of synovectomy or arthroplasty from knees, elbows, and hips of 32 patients with RA of 30 patients with no rheumatoid arthritis (osteoarthritis). The patients with rheumatoid arthritis fulfilled the 1987 revised criteria of the American College of Rheumatology (formerly, the American Rheumatism Association). EBV DNA was detected by PCR in synovial tissues from RA and NO-RA patients. Takeda et al. detected EBV DNA by PCR in none of those from the 30 NO-RA (no rheumatoid arthritis) patients but in 15 of the 32 samples from rheumatoid arthritis. The following table illustrates the data as obtained by Takeda et al.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Rheumatoid arthritis</th>
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<tbody>
<tr>
<td></td>
<td>EBV DNA</td>
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</tr>
<tr>
<td></td>
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<td>30</td>
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<td></td>
<td>32</td>
<td>62</td>
</tr>
</tbody>
</table>

Statistical Analysis
All statistical analyses were performed with Microsoft Excel version 14.0.7166.5000 (32-Bit) software (Microsoft GmbH, Munich, Germany). The method of the conditio per quam was used to proof the hypotheses: if EBV infection then rheumatoid arthritis. The mathematical formula of the causal relationship, and the chi-square distribution were applied to determine the significance of a causal relationship between a EBV infection and rheumatoid arthritis. A one-tailed test makes it much more easier to reject a null hypothesis (no causal relationship) while a two-tailed test makes it more difficult to reject a null hypothesis and is more conservative on this account.

Results

An Epstein-Barr virus infection is a conditio per quam of rheumatoid arthritis

Claims.

Null hypothesis:
An Epstein-Barr virus infection is not a main cause of rheumatoid arthritis. (k(EBV, RA) = 0).

Alternative hypothesis:
An Epstein-Barr virus infection is a main cause of rheumatoid arthritis. (k(EBV, RA) <> 0).

Conditions.
Significance level (Alpha two tailed) below which the null hypothesis will be rejected: 0.05.

Degrees of freedom: 1.
The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.
The data for this hypothesis test provided by Takeda et al. are illustrated in the 2 x 2 table. The causal relationship k(EBV, RA) is calculated as

\[ k_{\text{calc}}(\text{EBV, RA}) = \frac{(15 \times 30) - (0 \times 17)}{\sqrt{(32 \times 30) \times (15 \times 47)}} = 0.546993718 \]

The value of k(EBV, RA) is equivalent to a calculated chi-square value of
Discussion

Several study findings support the hypothesis that EBV is involved in RA disease pathogenesis. In contrast to healthy controls patients with existing RA have higher levels of antibodies against several EBV-encoded proteins, including VCA, EBNA-1, EBNA-2, and early antigen (EA). The EBV DNA load in peripheral blood mononuclear cells of patients suffering from rheumatoid arthritis is 10-fold elevated compared with the EBV DNA load in peripheral blood mononuclear cells in controls. The numbers of circulating EBV-infected B cells and EBV DNA loads in saliva are significantly higher in patients suffering from rheumatoid arthritis. In particular, several studies were able to provide some evidence that the levels of EBV DNA and mRNA are much higher in the synovium of patients with rheumatoid arthritis than in that of healthy controls. Takeda et al. were able to detect EBV in the synovial tissue of RA patients and concluded that EBV may be involved in the pathogenesis of RA. Still, one might argue that the very interesting study of Takeda et al. is based on a very small sample size n=62 patient and is of only limited value to detect large differences between designs or measures or to establish a causal relationship. In principle, bearing in mind the precision, statistical power and validity limitations of trials with small sample sizes, there is nothing wrong with conducting a well-designed small study. The technical quality of the study of Takeda et al. is very high. Takeda et al. used southern blot hybridization and/or polymerase chain reaction (PCR) amplification to detect EBV DNA. However, Takeda and all these other observations noted above have never been able to establish a cause effect relationship between Epstein-Barr virus and rheumatoid arthritis.

This study showed that there is a significant and an extremely high condito per quan relationship (p(EBV→RA)=1) between Epstein-Barr virus and rheumatoid arthritis. Together with the establishment of a condito per quan relationship between Epstein-Barr virus and rheumatoid arthritis, our present study indicates that not only Epstein-Barr virus implicates rheumatoid arthritis but Epstein-Barr virus is a cause of rheumatoid arthritis.

Conclusion.

A main cause of rheumatoid arthritis, a systemic and highly disabling autoimmune disease, no longer remains unknown. Highly significant evidence points to Epstein-Barr virus as a main cause of rheumatoid arthritis.

Conflicts of Interest:

The author declares that there are no competing interests.

References

virus infection increases the risk of rheumatoid arthritis in individuals with the shared HLA-DR4 epitope. Arthritis Rheum 1999;42:1485–96.