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

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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[2]. The prevalence of rheumatoid arthritis in men is twofold to fourfold 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 [6,7]. 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 [31,32,33], a cause or the cause of rheumatoid arthritis, a highly disabling systemic autoimmune disease, remains unknown.

Material and methods

Study design

Takeda et al. [34] 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) [35]. 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 1.		Rheumatoid arthritis		
		yes	no	
EBV DNA	yes	15	0	15
	no	17	30	47
		32	30	62

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[36] was used to proof the hypotheses: if EBV infection then rheumatoid arthritis. The mathematical formula of the causal relationship[37] k and the chi-square[38] 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 a conditio per quam of rheumatoid arthritis.

$p_0(EBV \rightarrow RA) \geq p_{critical}(EBV \rightarrow RA)$.

Alternative hypothesis:

An Epstein-Barr virus infection is not a conditio per quam of rheumatoid arthritis.

$p_0(EBV \rightarrow RA) < p_{critical}(EBV \rightarrow RA)$.

Conditions.

Significance level (Alpha) below which the null hypothesis will be rejected: 0.05

Proof.

The data as obtained by Takeda et al. [34] are viewed in the 2 × 2 table. The proportion of successes of a conditio per quam relationship between EBV and RA is calculated [36] as

$$p_{calculated}(EBV \rightarrow RA) \equiv \frac{(15+17+30)}{62} = \frac{62}{62} = 1$$

The critical value p_{lower} (significance level alpha = 0.05) is calculated [36] approximately as

$$p_{lower} \equiv 1 - \frac{3}{62} = 0{,}951612903$$

The critical value $p_{lower} = 0{,}951612903$ and thus far less than the proportion of successes calculated as $p_{calculated}(EBV \rightarrow RA) = 1$. Consequently, we cannot reject the null hypothesis in favour of the alternative hypotheses. The data as published by by Takeda et al. [34] do support our Null hypothesis that an Epstein-Barr virus infection is a conditio per quam of rheumatoid arthritis.

An Epstein-Barr virus infection is a main cause 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. [34] are illustrated in the 2 × 2 table. The causal relationship k(EBV, RA) is calculated [36, 37] as

$$k_{calc}(EBV, RA) = \frac{(15 \times 30) - (0 \times 17)}{\sqrt[2]{(32 \times 30) \times (15 \times 47)}} = 0{,}546993718$$

The value of $k_{calc}(EBV, RA)$ is equivalent to a calculated [37] chi-square value of

$$\chi^2_{calc} = n \times k_{calc}(EBV, RA) \times k_{calc}(EBV, RA)$$

$$\chi^2_{calc} = 62 \times 0{,}546993718 \times 0{,}546993718$$

$$\chi^2_{calc} = 18{,}55053191$$

The calculated chi-square statistic itself, uncorrected for continuity, is 18,55053191 and equivalent to a P value of 0,00001655. The calculated chi-square statistic is 18,55053191 and exceeds the critical chi-square value of 3.841458821. The data of Takeda et al. [34] do not support our null hypothesis. Consequently, we must reject the null hypothesis and accept the alternative hypothesis. In general, there is a highly significant causal relationship between an Epstein-Barr virus infection and rheumatoid arthritis (k = +0,546993718, p-value = 0,00001655). A sample size which is too small may fail to detect what is intended to do. A study based on a very large sample can waste unnecessarily more resources in the form of money, manpower, materials and time than needed to detect what is intended to do. Thus far, according to the central limit theorem as sample size of at least 30 samples is needed for a sample mean to be normally distributed. In this context we can state that about 100*(15/32) = 47% of rheumatoid arthritis is caused by Epstein-Barr virus. Altogether, an Epstein-Barr virus infection is a main cause of rheumatoid arthritis.

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 [39] , EBNA-1 [39,40,41,42] , EBNA-2 [43], and early antigen (EA) [39]. 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. [44]. The numbers of circulating EBV-infected B cells [45] and EBV DNA loads in saliva [46] is 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 [31, 33, 34, 40]. Takeda[34] 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. [34] is based on a very small sample size of n=62 patient and is of only of 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. [34] is very high. Takeda et al. used southern blot hybridization and/or polymerase chain reaction (PCR) amplification to detect EBV DNA. However, Takeda[34] et al. 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 conditio per quam relationship (p(EBV→RA)=1) between Epstein-Barr virus and rheumatoid arthritis. Together with the establishment of a conditio per quam 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 (k=+0,546993718, p-value = 0,00001655). About 100*(15/32) = 47% of rheumatoid arthritis is caused by Epstein-Barr virus. Consequently, Epstein-Barr virus, a DNA-containing herpesvirus which is infecting more than 98% of the human population by the age of 40 years [47] and which is extremely prevalent worldwide, is not the cause of rheumatoid arthritis but a main cause (k=+0,546993718, p-value = 0,00001655) 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.

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