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Leader

Rheumatoid arthritis and Epstein-Barr virus: a case of living with the enemy?

The cause of rheumatoid arthritis (RA) still eludes us, though we know from twin studies that both genetic and environmental factors are important contributory components to disease susceptibility[1]; the latter is estimated to account for about one half of this risk.[2] At least one major RA susceptibility gene resides within the major histocompatibility complex (MHC) region. Current dogma is that this is explained by a conserved sequence of amino acids within the third hypervariable region of the DRB1 β chain molecule encoded by a number of alleles. This is usually referred to as being the RA shared epitope hypothesis.[3] Although DRB1 molecules present peptide fragments to T cell receptors on CD4 positive lymphocytes, the exact mechanism through which the RA shared epitope exerts its effect remains unclear.[4] Given that class II molecules such as DRB1 serve an immunoregulatory role, it is not surprising that polymorphisms within these structures will influence variation in immune response in both health and disease states. It is likely that the RA shared epitope conveys disease susceptibility through its interaction with the environment. Characterising such interactions will be fundamental to our understanding of RA aetiopathogenesis.

A specific environmental/infectious trigger(s) for RA has yet to be identified, though there has been no shortage of contenders for this role, including mycoplasmas, parvovirus B19, cytomegalovirus, herpes virus 6, and Epstein-Barr virus (EBV).[5–7] The involvement of EBV in RA has been investigated and speculated about for over 15 years. Although definite proof is lacking, an increasing body of circumstantial evidence points at a close relation between the two. This evidence has been added to by the study conducted by Toussirot and colleagues (presented in this issue, p 533). These workers show that patients with RA have decreased T cell precursor frequencies to EBV gp110 (a late stage regulatory protein, sometimes also referred to as BALF 4) in peripheral blood and that this correlates with disease activity and severity.

This is a carefully performed study in which an attempt has been made to match HLA-DRB1 in patients with RA and controls. A lower T cell proliferative response was found in patients than controls for gp110 but not for a total protein extract from *Escherichia coli*, showing that this reduction in response is specific rather than generic. Interestingly, no difference was found between shared epitope positive and negative cases, though a lower level was apparent in patients with severe disease, especially in those with extra-articular disease. The conclusions drawn from these findings are that such decreased immunity to an important EBV regulatory protein might lead to poor control of EBV infection (or re-infection), chronic exposure to other EBV antigens, and the development of chronic inflammatory responses in RA. It is important to consider these findings in the context of what we already know about EBV and its possible role in RA.

EBV is a γ herpes virus and a ubiquitous pathogen. Although not everyone has a documented clinical history of infectious mononucleosis, about 95% of adults have positive serological evidence of infection. This indicates that a range of clinical infection exists, with many subjects having unnoticed subclinical infections. EBV initially infects oropharyngeal epithelial cells but also targets B lymphocytes in which it establishes a reservoir of latently infected cells. It can also infect synovial fibroblasts,[8] and poymerase chain reaction based studies have detected EBV DNA in the synovial tissues of patients with RA.[9] Sterile immunity to EBV is probably never achieved and there is a lifelong persistence of the virus largely in a latent form. The persistence of viral infection is the result of alternate phases of production (lytic) and non-production (latent). Periodic reactivation of latent infected B lymphocytes can lead to secondary foci of viral replication.

The control of long term virus carriage and its reactivation is largely regulated by host levels of the immune response. Although antibody levels to EBV antigens are likely to contribute to this process, there is evidence that CD8 cytotoxic T cells are primarily the way in which viral carriage is kept in check.[10] This is corroborated by the high prevalence of EBV associated lymphoproliferative conditions in immunosuppressed or compromised subjects. Interestingly, implication of EBV in cases of methotrexate induced lymphoma has been reported.[11] Evidence has been presented to show that persistent infection with EBV causes major distortions within the memory repertoire of healthy virus carriers.[12] The functional significance of dramatic clonal expansions in healthy adults can be linked in some cases to virus-specific CD8 T cells that have an essential role in immunosurveillance.

Considerable circumstantial evidence has accumulated implicating but not proving an involvement of EBV in RA. Increased titres of antibodies to EBV antigens have been shown in patients with RA.[13] Furthermore, higher viral loads of EBV have been reported in patients with advanced RA.[14] However, an increased prevalence of EBV in the joints of patients with RA compared with controls remains controversial, and studies have reported conflicting observations.[9 15–17] Thus a direct causal link has not yet been categorically established and, possibly, the above

sequelae seen in patients with RA are "effect" rather than "cause" and can be explained by an underlying immune dysregulation in patients with RA.

Increased levels of intrasynovial CD8 T cells which can recognise EBV derived epitopes have been seen in patients with RA.[14] This presents a paradox as it suggests that immune mediated control in patients with RA should be enhanced and this does not fit with clinical or laboratory observations. Poor immune regulation of EBV is apparent in both patients with RA and Sjögren's syndrome.[18] This deficiency appears to lie in the T cell compartment as B lymphocytes from patients with RA can be relatively easily immortalised in vitro into cell lines with EBV even when autologous T cells are present. However, if T cells are added from an HLA identical, healthy sibling to B lymphocytes from a patient with RA, this process is much more difficult to achieve.[19] Conversely, B lymphocytes from the healthy sibling will EBV transform more easily when T cells from the HLA identical RA sibling are added.

Polymerase chain reaction has been used to investigate the rate and extent of infection by EBV, cytomegalovirus and herpes virus 6 in families containing multiple cases of RA.[20] Viral DNA was detected in cells from saliva and peripheral blood; this was particularly the case for EBV, which was found in increased prevalence in patients with RA compared with their non-affected relatives. This clearly establishes a relation between EBV and RA but does not prove a direct causality. Similarly, EBV DNA and mRNA transcripts have been found to be more common in synovial tissues of patients with RA than in controls.[17] This correlates with the patient's HLA-DR genotype; subjects with EBV detected in their synovial tissue and who are HLA-DR4 or RA shared epitope positive had a markedly increased risk of RA. It should be added, however, that not all studies have shown such a marked increase in EBV DNA[15] or gene expression[16] in the synovial tissue of patients with RA.

Considerable interest has been generated by the observation that gp110 EBV viral protein contains a sequence of amino acids (QKRAA) which corresponds to the third hypervariable region of HLA-DRB1 alleles associated with RA risk.[21 22] The RA shared epitope sequence has also been identified in proteins from a number of other prokaryotes, including *E coli*, *Brucella ovis*, and *Lactobacillus lactis*.[23] This has formed the basis for a molecular mimicry hypothesis to explain RA aetiology. T cells positively selected in the thymus by low affinity interactions with self MHC peptides may later be triggered in the periphery upon exposure to foreign peptides similar enough to cross react and break immunological tolerance.[24 25] Further cross reactivity with synovial membrane components might then lead to an autoimmune driven pathology.

Previous studies have shown greater antibody levels to EBV gp110 in patients with RA than in controls.[23] This is not the case when sera are tested against gp110 where the QKRAA sequence has been experimentally deleted, suggesting that this represents a major or dominant epitope recognised in patients with RA. Similarly, increased T cell proliferative responses to EBV gp110 containing the QKRAA sequence were found for shared epitope positive patients with RA compared with controls and with shared epitope negative patients with RA.[23] These data seem to be at odds with those presented by Toussirot, where lower T cell precursor features were seen in patients with RA than in HLA matched controls. This apparent difference has been explained by different stages of disease in the patients studied, though this seems unlikely. An earlier study by the same group also reported that HLA-DR polymorphism influenced T cell precursor frequencies to EBV gp110 in

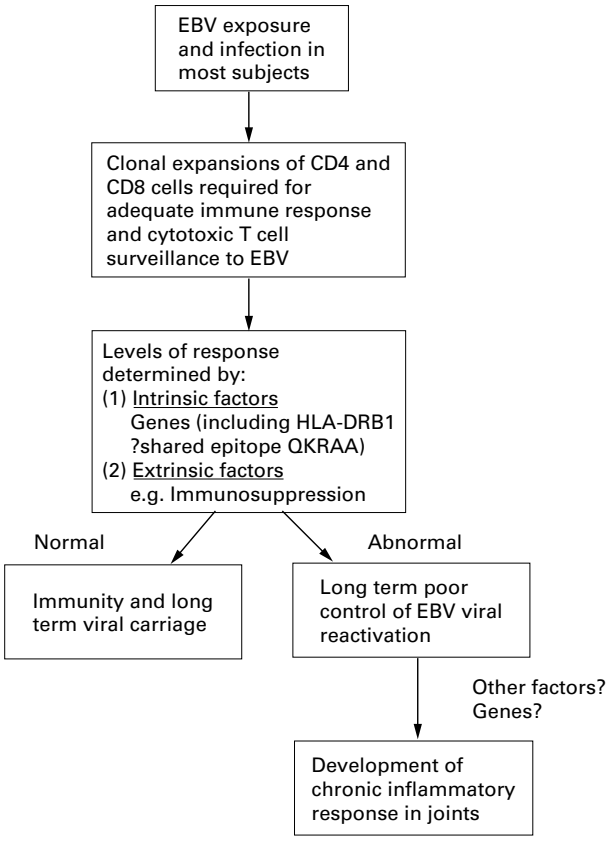

Figure 1 A possible model for Epstein-Barr virus (EBV) in rheumatoid arthritis.

healthy subjects[26]; shared epitope positive status was marginally associated with lower T cell precursor frequencies than shared epitope negative status. As T cell precursor frequencies are used as a measure of potential T cell proliferative capacity, these conflicting results suggest that we still need to re-examine the relation between HLA-DR, the QKRAA sequence and EBV gp110.

Although it remains unclear whether increased levels of EBV in patients with RA are cause or effect, it does seem that this is a phenomenon not related to other commensal latent viruses. Even if increased activation of EBV in patients with RA is due to an underlying genetic dysregulatory mechanism in the immune response, the virus could have a significant role in RA joint disease in a number of different ways.

EBV has evolved to modulate host immune responses by encoding a homologue of human interleukin 10 (IL10) in its sequence. This is known as viral IL10 and can suppress T helper 1 (TH_1) responses, but it may lack some of the immunostimulatory properties of IL10. This presumably assists in maintaining viral infections by damping down T cell immunity. Such effects would be expected to be beneficial in RA by suppressing cell mediated processes in the synovium. Indeed the potential for using EBV viral IL10 in gene therapy for RA is presently being explored.[27] However, EBV infected synovial and B cells could participate in RA pathology in other ways. Recently it was reported that human IL6 expression in rheumatoid fibroblast-like synoviocytes can be transcriptionally regulated by Epstein-Barr C promoter binding factor 1.[28] Given the likely involvement of IL6 in RA pathology this could be an important aspect involving EBV. Other recent studies have also shown that EBV infected B cells and plasma cells can secrete matrix metalloproteinases[29] and the proinflammatory cytokine, tumour necrosis factor α,[30] These factors

are key players in RA joint disease and if EBV, for whatever reason, is more prevalent in RA synovium, it might help to drive the inflammatory response. Cross reactivity between self joint specific antigens and EBV encoded peptides has not been clearly shown for T cell epitopes, though this is not the case for B cell epitopes. Phage display techniques have identified mimotopes for a conformational epitope of type II collagen and shown an interesting homology with a sequence of Epstein-Barr nuclear antigen 1.[31]

Infection of B lymphocytes with EBV induces the production of a new host protein (EBI3-EBV induced gene 3).[32] This protein is related to the p40 subunit of IL12, a cytokine which can induce TH_1 responses and proliferation through stimulation of interferon γ. This structural similarity suggests that secreted EBI3 might antagonise the effects of IL12. EBI3 could represent a mechanism by which EBV overcomes cytotoxic T lymphocyte reactivity to viral antigens. When secreted it might lead to expansion of latently infected cells by interfering with IL12 activity.[33] This provides a further strategy employed by EBV to affect IL12 as viral IL10 also inhibits IL12 synthesis.

In summary, Toussirot and colleagues have provided further evidence to implicate EBV in RA. However, their findings are at odds with a number of earlier reported observations and to some extent a muddy pool has been stirred up even more. A causal link between EBV and RA still cannot be supported, but it does seem increasingly likely that viruses such as EBV have a role in the progression or exacerbation of inflammatory responses within the RA joint. Recently it has been shown in vitro that retinoids can limit the proliferation and differentiation features of EBV.[34 35] If treatments can be developed which limit or avoid reactivation of EBV, these may be beneficial in RA.

Conjecture about a role for EBV has been about for many years and, like its clinical course in humans, it periodically emerges to the fore in rheumatology only to again subside. Because most of us live quite happily with our EBV we do not afford it the respect that it perhaps deserves.

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