Herpes viruses are a large family of agents whose members have long coevolutionary histories with their natural hosts. The product of that coevolution is a virus-host balance typified by asymptomatic or clinically mild primary infection, followed by virus persistence through latent infection of a specific cellular niche; thereafter, occasional asymptomatic reactivation from that niche allows transmission of infectious virions from the lifelong carrier to a new naive host. In certain circumstances, this evolutionary compact between virus and host can be disturbed and the full pathogenic potential of these agents realized. One such circumstance highlights the important role that host immunity has played in shaping virus-host coevolution; thus, herpes virus infections can be life threatening in transplant recipients whose cellular immune function has been impaired. Another such circumstance highlights the fact that virus and host have coevolved to accommodate primary infection occurring very early in life, once passively acquired maternal antibodies have waned; thus, changing the natural epidemiology of infection may also affect its outcome. An example involves Epstein–Barr virus (EBV), a B-lymphotropic human gammaherpesvirus that is normally acquired by oral transmission from close family contacts early in life, at which time primary infection is almost always asymptomatic. Over the last century, in economically developed countries with rising standards of human hygiene, primary infection has become increasingly delayed until adolescence or later. In these circumstances, a proportion of persons present clinically with infectious mononucleosis (IM), an acute illness characterized by a quartet of symptoms and signs (sore throat, cervical lymphadenopathy, fever, and fatigue) that can range from mild to extremely severe in intensity and duration. For some time now, investigators have been wrestling with 3 interrelated questions: (1) Exactly what proportion of delayed primary infections is symptomatic? (2) Why are some delayed infections symptomatic and others not? and (3) What is different about acquiring the virus in adolescence rather than in infancy? The report by Balfour and colleagues [1] in this issue of the Journal describes the most thorough investigation to date addressing the first of these questions, and it provides further evidence to frame our thinking about the second and third.

As in previous studies, these investigators screened college entrants for virus-specific antibodies to identify a study cohort (in this case, 37% of all entrants) who were still EBV naive and, therefore, potentially at risk of primary EBV infection, with or without IM symptoms. What distinguishes this study from earlier work is the rigor of the prospective follow-up, involving 143 participants who provided both blood and throat washing samples roughly every 8 weeks over 4 years and donated additional samples in the event of any febrile illness occurring during that time. A total of 66 individuals at risk acquired EBV, as defined by development of immunoglobulin G antibodies to virus capsid antigen, in that 4-year period, and the incidence of acquisition was 2-fold higher in the first year than in later years. Very interestingly, 51 of 66 serologically confirmed primary infections (77%) were clinically manifest as IM, defined in this study by the presence of at least 2 of the quartet of symptoms and signs specified above. A further 8 cases were symptomatic but did not meet the above criteria, thereby raising the total incidence of symptomatic infections to 89%. Such figures are significantly higher than the 25%–50% incidence of IM reported in most other prospective studies of this kind [2–5]. This increase in recorded symptomatic infections likely reflects Balfour et al’s much more frequent monitoring of the study cohort and their assiduous attention to clinical detail. As a result, this article is likely to become the definitive

Epstein–Barr Virus and Infectious Mononucleosis: What Students Can Teach Us

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(See the major article by Balfour et al, on pages 80–8.)
study identifying the true burden of acute disease caused by delayed primary infection in otherwise healthy, nonimmunocompromised individuals.

That said, questions still remain as to what determines symptom occurrence/ severity and, in particular, what distinguishes early from late infections. In this latter context, Balfour et al address the notion that primary infections among adolescents and young adults may be mediated not just by the usual oral route but also by genital transmission through sexual intercourse [6, 7], low levels of EBV DNA having been detected by polymerase chain reaction (PCR) in at least a proportion of cervical and urethral samples from virus carriers [8, 9]. However, their data firmly contradict this notion and are entirely consistent with all late virus acquisitions (silent or symptomatic) occurring via the oral route; thus, students with no history of kissing remained seronegative, whereas students with a history of deep kissing showed similar conversion rates whether or not they had had penetrative sexual intercourse.

A major bonus from the regular screening of this EBV-naïve cohort was that, in many cases, whole blood, peripheral blood mononuclear cell (PBMC), and throat washing samples were taken from individuals ≤50 days before IM or IM-like symptoms developed, which is within the estimated 4–7-week incubation phase of the disease [10]. In most such presymptomatic cases, viral DNA was undetectable in either whole blood or throat washings; indeed, levels were also undetectable or low in those few individuals sampled in the potentially most interesting period, within 10 days of symptoms. Although there are caveats about the sensitivity of the PCR assay being used, it is nevertheless clear that presymptomatic EBV genome levels were well below those detected during disease. This argues against massive lytic EBV replication in the throat or massive expansion of latently infected B cells in the blood during the incubation phase, although it does not necessarily inform on events occurring within pharyngeal lymphoid tissues [11, 12].

Likewise, the large expansions of activated CD8+ T cells that are characteristic in blood from individuals with acute IM were not apparent before symptoms appeared, although there were hints that increased natural killer (NK) cell numbers, another feature of acute IM, sometimes preceded disease onset. Such hints might reflect NK cell detection of early foci of virus replication, since HLA class I downregulation sensitizes lytically infected cells to NK cell recognition in vitro [13]; indeed, others have speculated on a role for NK cells in the early restraint of EBV infection, on the basis of an inverse correlation between circulating NK cell numbers and virus load in the blood of IM patients [14]. However, Balfour et al did not confirm this; in their IM patients, both NK cell numbers and CD8+ T-cell numbers showed significant positive correlations with viral load in the blood. One has to be cautious in extrapolating from statistical to biological significance with data of this kind, particularly when the viral load is falling rapidly, because the time of sampling after symptom onset can be critical; nevertheless, the results do suggest that both NK cells and CD8+ T cells are responding similarly to the intensity of viral challenge. And here is the paradox. There are strong reasons to believe that both of these arms of the cellular response are largely driven by direct interactions with infected B cells [15–17]; furthermore, given the nature of NK cell recognition (see above) and the well-documented lytic antigen focus of the CD8+ cell response [18], both appear to be chiefly directed against lytic rather than latent infection. However, by the time IM symptoms appear, virus genome–positive B cells in blood and tonsillar tissues are almost all latently infected [11, 18]. How can this be explained? Perhaps there is an initial phase of full virus replication in oropharyngeal B cells (before latent infections become dominant), and this phase drives the cellular response. Thereafter, the site of virus replication likely transfers to permisive oropharyngeal epithelium, leading to high-level virus shedding in the throat that, as both Balfour et al and others have shown [19, 20], continues long after the blood picture normalizes and IM symptoms resolve. Thus, in primary infection as in the lifelong carrier state [21], there is a temporal disconnect between patterns of lytic infection in the throat and latent infection in the B cell system, as if the 2 entities are subject to different host controls.

Many believe that IM is an immunopathologic disease, in which the symptoms result not from virus infection of the B cell system per se but from the host’s exaggerated CD8+ T cell and/or NK cell response with its attendant cytokine release. However, this remains unproven and, in showing that the severity of symptoms in their patients correlates positively with viral load in the blood and with the degree of CD8+ T-cell expansion, Balfour et al could not disentangle the two. Furthermore, the only cytokine in plasma from subjects with IM whose level showed any correlation with symptom severity was interleukin 6, and this may derive from infected or reactive cell types. Resolving the pathogenesis issues will need more data from asymptomatic primary infections, of which, ironically, there were too few in Balfour et al’s cohort to draw firm conclusions. Elsewhere, the evidence is limited. In one early study, 2 of 4 asymptomatic adult infections were identified with very high virus loads in the blood, yet, by T-cell receptor analysis, both lacked the skewed Vbeta repertoire typically produced by the large EBV-specific CD8+ T-cell expansions seen in IM patients [22]. Again, in more recent studies, infants in Africa acquire the virus apparently asymptomatically, yet they exhibit very high PBMC viral loads [23, 24]; furthermore, such infants can mount individual EBV epitope-specific responses that, by tetramer staining, amount to 5%–15% of the CD8+ T-cell
pool, but the CD8+ population as a whole is not expanded to IM-like levels (Jayasooriya et al, unpublished data). Such data suggest that symptomatic infection is linked neither to virus load nor to the quality of the virus-induced T-cell response, but to its overall size. Why responses to delayed infection should be so expanded remains to be determined. One suggestion is that, with age, one accumulates a more diverse pool of CD8+ memory cells from which T-cell clonotypes with fortuitous EBV cross-reactivity can be recruited into the primary response to EBV infection [25]. However, this seems unlikely to be the whole story, not least because of Balfour et al’s own tetramer findings [26]. These continuing uncertainties only serve to emphasize how much there is still to learn about the biology and immunology of this virus and its linkage to disease. And as the heroic efforts of Balfour et al make clear, if one wants to understand the factors predisposing to IM, students can teach us a lot!

Notes

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