expert reviews
in molecular medicine

Infectious mononucleosis and Epstein–Barr virus

Eleni-Kyriaki Vetsika and Margaret Callan

Epstein–Barr virus (EBV) is a γ-herpesvirus that infects over 90% of the human population worldwide. It is usually transmitted between individuals in saliva, and establishes replicative infection within the oropharynx as well as life-long latent infection of B cells. Primary EBV infection generally occurs during early childhood and is asymptomatic. If delayed until adolescence or later, it can be associated with the clinical syndrome of infectious mononucleosis (also known as glandular fever or 'mono'), an illness characterised by fevers, pharyngitis, lymphadenopathy and malaise. EBV infection is also associated with the development of EBV-associated lymphoid or epithelial cell malignancies in a small proportion of individuals. This review focuses on primary EBV infection in individuals suffering from infectious mononucleosis. It discusses the mechanism by which EBV establishes infection within its human host and the primary immune response that it elicits. It describes the spectrum of clinical disease that can accompany primary infection and summarises studies that are leading to the development of a vaccine designed to prevent infectious mononucleosis.

Infectious mononucleosis (IM) has been recognised as a clinical entity for over a century. It can result from primary infection with one of several organisms including Epstein–Barr virus (EBV), cytomegalovirus and *Toxoplasma*. This review focuses on the association between EBV and IM, and describes recent advances in understanding the biology of primary EBV infection, the immune response that it elicits, and the clinical syndrome that can accompany primary infection.

Eleni-Kyriaki Vetsika
Research Associate, Division of Medicine, Imperial College, Chelsea and Westminster Hospital, 369 Fulham Road, London, SW10 9NH, UK. Tel: +44 (0)20 8746 8421; Fax: +44 (0)20 8746 8030; E-mail: e.k.vetsika@imperial.ac.uk

Margaret Callan (corresponding author)
Professor of Immunology and Rheumatology, Division of Medicine, Imperial College, Chelsea and Westminster Hospital, 369 Fulham Road, London, SW10 9NH, UK. Tel: +44 (0)20 8746 8421; Fax: +44 (0)20 8746 8030; E-mail: m.callan@imperial.ac.uk

Institute URL: http://www.ic.ac.uk

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

EBV discovery and structure

Discovery and disease

EBV was first identified in 1964 by Anthony Epstein and his PhD student Yvonne Barr, as a result of electron microscopy of cultured cells derived from Burkitt's lymphoma (Ref. 1). It was not until four years later, when a laboratory worker seroconverted to EBV after suffering from IM, that the virus was implicated as the cause of this infectious disease (Refs 2, 3, 4). Subsequent sero-epidemiological studies have shown that the majority of individuals are persistently infected with EBV (Ref. 5). Seroconversion usually occurs during early childhood and is not associated with important clinical symptoms (Refs 6, 7). However, if primary exposure to the virus is delayed until adolescence or adult life, it can cause IM in a proportion of individuals (Refs 8, 9). In addition, persistent infection can be associated with the development of malignancies of lymphoid or epithelial cells in a very small minority of individuals (reviewed by Refs 10, 11, 12). Since its discovery, EBV has been studied intensively, and enormous progress has been made towards understanding the mechanisms by which the virus infects and persists within its human host.

Virus structure

EBV, also known as human herpesvirus 4, is a member of the γ-herpesvirus family. In common with the other herpesviruses, EBV has a protein core that is wrapped with DNA. This, in turn, is surrounded by a nucleocapsid, the protein tegument and the outer envelope with glycoprotein spikes. The complete DNA sequence of the EBV B95-8 genome was first published in 1984 (Ref. 13). The genome comprises linear double-stranded DNA of approximately 172 kb. It includes highly repetitive terminal repeats as well as internal repeats that divide up the largely unique sequence domains. Over 70 different open reading frames have been identified within these sequence domains. The genes are known according to their position on a *Bam*H1 restriction endonuclease map of the genome.

Forms of EBV infection

EBV expresses different sets of genes, and hence proteins, in order to establish different types of infection within its human host (Ref. 14). All the lytic cycle proteins are expressed in the replicative form of infection; these are classified into 'immediate-early', 'early' and 'late' lytic proteins. All the latent genes are expressed in the latency III or 'growth' programme of infection; these include the leader protein (LP), Epstein–Barr nuclear antigens (EBNAs) 1, 2, 3A, 3B and 3C, and the latent membrane proteins (LMP) 1, 2A and 2B (see Fig. 1 in Ref. 15 for a diagram in this journal showing the genomic organisation of the latency genes). Noncoding RNAs termed the EBERs are also expressed. A detailed description of the functional properties of the EBV latent proteins is beyond the scope of this work and can be found

Table 1. Patterns of Epstein–Barr virus (EBV) gene expression in forms of latent infection identified in different EBV-associated malignancies

Feature	Latency I	Latency II	Latency III
Gene expression			
EBERs	+	+	+
EBNA1	+	+	+
LMP1		+	+
LMP2		+	+
EBNA2			+
LP			+
EBNA3s			+
EBV-associated malignancy	Burkitt lymphoma	Hodgkin lymphoma Nasopharyngeal carcinoma	Post-transplant lymphoproliferative disease

Abbreviations: EBER, Epstein–Barr virus encoded small RNAs; EBNA, Epstein–Barr nuclear antigen; LMP, latent membrane protein; LP, leader protein.

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

in other recent reviews (e.g. Ref. 14). However, it is important to note that EBNAs 2, 3A and 3C and LMP1 can act as oncogenes (Refs 15, 16, 17, 18, 19). Certainly, expression of the full panel of latent proteins in the 'growth' programme allows the virus to drive the activation and proliferation of infected cells (Ref. 19). A more restricted set of latent proteins is expressed in other forms of latent infection. These types of infection were first recognised in the context of EBV-associated malignant disease (Table 1) (reviewed by Ref. 15); however, recent studies suggest that EBV might also normally use these different forms of latent infection in the course of establishing infection and persisting within its human hosts (Refs 20, 21, 22, 23, 24).

EBV infection and persistence

EBV is usually transmitted between individuals orally, in saliva, and establishes lytic infection within the oropharynx. Although the virus is able to infect epithelial cells, there is some doubt as to whether this is a feature of natural primary infection, since EBV infection of tonsillar epithelial cells in patients with symptomatic primary infection has not yet been demonstrated (Ref. 25). By contrast, both lytic and latent infection of local B cells has been shown to occur and it is possible that EBV directly accesses this lymphoid compartment via the tonsillar crypts (Ref. 26).

A model for infection and persistence within B cells; lessons from persistent infection

The details of the mechanism by which EBV establishes infection and persists within the B-cell compartment following primary exposure to the virus in patients with IM remain controversial. However, an elegant hypothesis for virus infection and persistence has been developed from work performed using tonsils taken from healthy EBV-seropositive individuals (Refs 20, 21, 22, 23, 24). The model suggests that EBV infects naive B cells and that it exploits different forms of latent infection in order to drive B cells through a maturation process that mimics the progression of a B cell through a primary antigen-driven response (Fig. 1).

It is proposed that initial infection of naive B cells will result in all viral latent proteins being expressed (i.e. latency III programme), thereby allowing the virus to drive the activation and perhaps proliferation of the naive B cells. These cells would migrate to germinal centres (GCs) – discrete lymphoid compartments where B cells divide, mature and become memory cells, which are found in all peripheral lymphatic tissues, including the tonsil. Within the GC, the B cells would downregulate expression of EBNA2, LP and the EBNA3s. Continued expression of the LMPs (latency II programme) would allow the B cells to receive signals required for survival in a GC reaction; LMP2a might mimic signalling through the B-cell receptor and LMP1 might provide signals similar to those induced by CD40 engagement (Refs 27, 28, 29, 30). These EBV-infected B cells could then exit from the GC reaction as resting memory B cells and, at this stage, would downregulate expression of virtually all viral proteins (Ref. 31). EBV could thereby persist within the peripheral resting memory B-cell pool and effectively evade the host immune response. The viral genome is not only able to persist within these peripheral resting memory B cells, but can be transmitted to each of the daughter B cells following host cell division. This is achieved by expression of EBNA1 (latency I programme) within dividing cells; this DNA-binding protein serves to maintain the virus genome during host cell division (Ref. 31). Alternatively, in B cells that recirculate to the tonsillar region, a switch into the virus lytic cycle might occur, perhaps triggered by maturation of the B cells to plasma cells. This then allows for virus replication, shedding of virus into saliva and transmission to new hosts. Newly formed virus might also infect further naive B cells within the same host.

EBV infection and persistence within tonsillar B cells following primary exposure in patients with symptomatic infection

Parallel studies investigating EBV gene expression with similar methodology have not yet been performed using tonsils taken from patients with IM. However, detailed studies of the subtypes of B cells within the tonsils that are infected with EBV have been published and the data appear to be conflicting in some respects (Refs 32, 33, 34). During symptomatic primary infection, EBV can be found within some naive B cells but resides predominantly within GC or memory B cells identified on the basis of their immunoglobulin variable gene mutations. Further analysis reveals that the EBV-infected B cells that are clonally expanded are GC or memory B cells.

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

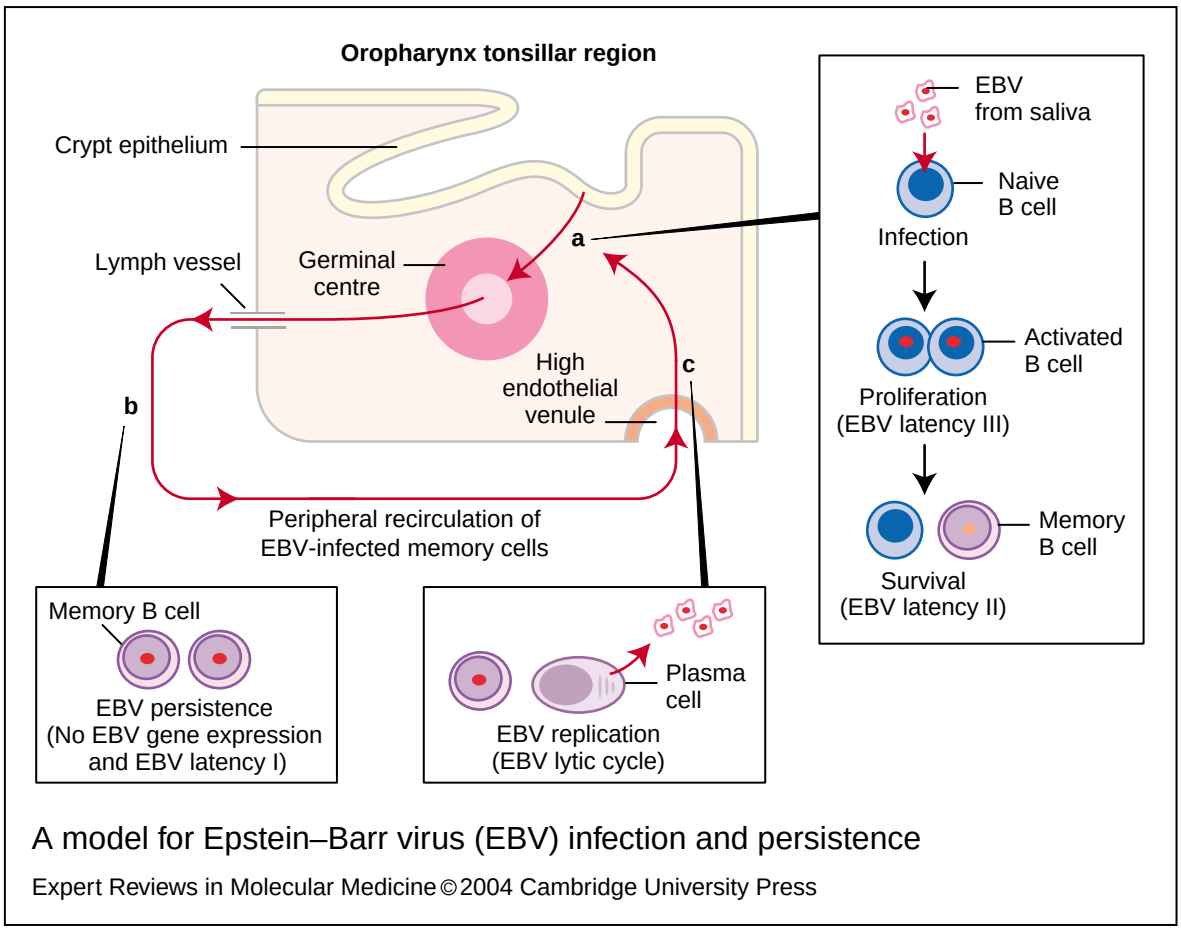


Figure 1. A model for Epstein–Barr virus (EBV) infection and persistence. (a) In the oropharynx, EBV infects naive B cells and expresses the full spectrum of latent proteins (latency III programme: EBNA1, LMPs 1 and 2A, EBNA3s and LP). The virus can thereby drive the activation and proliferation of these B cells, which then migrate to lymphoid follicles and form germinal centres. Concomitantly, expression of EBNA3s and LP are downregulated, leaving EBNA1 and the LMPs expressed (latency II programme). Expression of the LMPs provides signals that allow the B cells to survive the germinal centre reaction and form resting memory B cells. (b) These resting memory B cells exit to the periphery. At this point, expression of other EBV proteins is downregulated, thereby allowing the virus to persist within the B cells but to evade a host immune response. Intermittent expression of EBNA1 within dividing B cells allows the virus genome to be distributed to each of the daughter B cells (latency I programme). (c) As B cells recirculate to the oropharynx, a switch into the EBV lytic cycle might occur, possibly triggered by maturation of B cells into plasma cells, allowing for virus replication, shedding into saliva and transmission both to new hosts and to previously uninfected B cells within the same host. This model is derived from work published by Thorley-Lawson and colleagues (Refs 11, 20, 21, 22, 23, 24, 31, 39).

There is currently no evidence to suggest that the expanding clones of EBV-infected B cells found in the tonsils during primary infection exhibit intraclonal sequence diversity. This implies that, during primary infection, EBV can infect both naive and GC/memory B cells but that it drives the proliferation of the GC/memory B cells preferentially. Furthermore, in contrast to studies that focus on healthy seropositive individuals, this work does not provide evidence to support the idea that EBV-infected B cells need to pass through a GC reaction to allow virus persistence in the B cells, at least in patients with IM.

One possible conclusion from the studies is that there are real differences in the biology of virus infection between IM and the persistent phase of infection. IM may be an atypical state in which the virus is able to infect GC and memory B cells directly and drive their proliferation – hence their predominance within the tonsillar

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

tissue. Such cells might not differentiate according to the model shown in Figure 1 and might need to be cleared by an appropriate immune response. This does not necessarily exclude the idea that virus infection of naive B cells drives their differentiation through to a resting memory B cell and that such cells are the source of the EBV-infected B cells in the periphery, although it does suggest that the virus-infected cells do not proliferate extensively during this process of differentiation.

EBV infection within circulating B cells in patients with symptomatic primary infection

The nature of EBV infection within circulating B cells in patients with IM has also been the subject of controversy. There has been dispute both as to whether the infected cells are lymphoblastic and as to the pattern of virus gene expression that predominates within these cells. Whereas Crawford et al. suggested that the infected cells were small, probably resting and expressed little or no EBNA (Ref. 35), most other early studies reported that the infected cells were B lymphoblasts and that the latency III pattern of gene expression predominated (Refs 36, 37). Furthermore, one study concluded that circulating B cells supported replicative infection (Ref. 38). The most recent evidence suggests that, even in IM, EBV infection is tightly restricted to the resting memory cell compartment and that the virus expresses very few, if any, latent proteins within the vast majority of these cells (Ref. 39). This new work also provides higher estimates of the numbers of B cells that are infected within the peripheral circulation in patients with IM. Although there was wide variation between different donors, with between 0.1% and 46% of B cells being infected, over 10% of circulating B cells carried the virus in a quarter of the donors studied.

After symptomatic primary EBV infection, the number of infected B cells within peripheral blood falls rapidly over the first few weeks and then more slowly over the course of months. Even 12 months after IM, the frequency of EBV-infected B cells within the peripheral circulation of patients was found to be higher than the mean of approximately 0.005% found in healthy EBV-seropositive individuals. The means by which these latently infected cells die is unclear; however, the lack of expression of viral proteins suggests that they should be protected from the immune response. One possibility is that they recirculate back to the tonsil, where they produce infectious virus and are thereby lysed or subject to immune attack.

The primary immune response to EBV infection

The primary immune response to EBV has, of necessity, been studied mainly in symptomatic individuals and thus the discussion below relates to patients with IM. One should not assume that similarly vigorous primary responses are seen in individuals who seroconvert asymptomatically. However, the overall importance of effective immunity during primary infection is highlighted by observations that primary EBV infection in immunocompromised individuals can be associated with uncontrolled infection and the development of B-cell lymphomas.

Innate immunity to EBV

The innate immune system generally provides the first protective response to a pathogen. Current interest is focusing on the role of natural killer (NK) cells in controlling early EBV infection. Published studies have shown that NK cells are capable of recognising and killing EBV-transformed B-lymphoblastoid cell lines, via the natural cytotoxicity receptor p46. CD48 on the B cells and 2B4 on the NK cells are important co-receptors in this interaction (Ref. 40). Patients with X-linked lymphoproliferative disease (XLP) are unable to control primary EBV infection. Such patients have a mutation in Src-homology-2-domain-containing protein (SH2D1A or SAP), a molecule that regulates signal transduction by 2B4 as well as by SLAM (signalling lymphocyte-activation molecule) (Ref. 41). In the absence of functional SH2D1A, 2B4 acts to inhibit rather than activate NK cell function and the NK cells do not kill EBV-transformed B cells (Refs 40, 41, 42, 43). Although SH2D1A also has an important influence over T-cell responses (Ref. 44), the defective NK cell immunity is likely to contribute to the inability of patients with XLP to control primary EBV infection. Studies investigating a role for NK cells in controlling primary infection in patients with IM or those who seroconvert silently are now needed. However, if the NK responses are an early feature of infection, then the inevitable delay between initial infection and diagnosis of primary EBV infection might preclude easy study of responses in vivo.

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

Humoral immunity to EBV

The antibody responses to EBV have classically been reported in terms of reactivity to a series of antigenic preparations: the EBNA, the early antigen (EA), the viral capsid antigen (VCA) and the membrane antigen (MA). Each of these antigenic preparations includes a range of EBV proteins. The EBNA includes LP as well as EBNAs 1–3, and the EA includes many of the immediate-early and early lytic cycle proteins. Reactivity to VCA might reflect antibodies specific for EBV nucleocapsid and envelope proteins including glycoproteins, whereas a response to MA is indicative of antibodies specific for the glycoprotein gp350.

Primary EBV infection, at least in individuals with the clinically associated syndrome of IM, is characterised by production of IgM heterophil (or Forssman) antibodies (Ref. 45), which react with glycolipids (Forssman antigens) found only on sheep red blood cells and not on the blood cells of other species. This is known as the Paul–Bunnell test, and is the classical test for detection of heterophil antibodies in IM (Ref. 46). Confirmation of the diagnosis of primary EBV infection may then usually be made on the basis of the presence of IgM antibodies specific for the VCA. IgG antibodies specific for VCA, EA and EBNA2 become detectable soon afterwards. IgG antibodies specific for the MA gp350 are neutralising but tend to arise fairly late in the course of primary infection. These, together with IgG antibodies specific for EBNA1 and VCA, are usually detectable within serum thereafter, during the life-long persistent phase of EBV infection.

$CD8^+$ T-cell immunity to EBV in patients with IM

Clonal expansions of $CD8^+$ T cells

IM is associated with a very striking peripheral lymphocytosis, predominantly reflecting a massive expansion of activated $CD8^+$ T cells. It was initially assumed that the bulk of this reflected a 'bystander' phenomenon – that is, activation and proliferation driven not by cognate antigen, but by other factors such as cytokines. However, the observation that the $CD8^+$ T-cell populations in patients with IM included clonal expansions suggested, for the first time, that much of the expansion might instead be antigen driven (Ref. 47). In some individuals, large clonal or tightly oligoclonal expansions comprising 30% of all circulating T cells were identified. In the majority, smaller expansions constituting 2–10% of $CD8^+$ T cells were found. Further work, using molecular tools, found multiple smaller clones were likely to account for at least some, if not the majority, of the remainder of the expanded $CD8^+$ T-cell pool (Ref. 48). Cells within the expansions expressed the R0 isoform of the phosphatase CD45 (CD45R0) and CD38, suggesting that they were antigen-experienced and -activated. In addition, the cells were heterogeneous with respect to expression of the costimulatory molecule CD28, and few expressed the glycoprotein CD57 that has been associated with cellular senescence. The cells were therefore different from the stable, $CD28^-$ $CD57^+CD8^+$ T-cell expansions that have been identified in elderly individuals (Ref. 49). Moreover, following resolution of the primary EBV infection, the expansions were transient and the T-cell receptor (TCR) repertoire returned to normal (i.e. there was no longer large clonal or oligoclonal expansion of certain $CD8^+$ T cells). The evidence is indirect but it strongly suggested that EBV drives a massive primary antigen-specific T-cell response in patients with IM.

Identification of EBV-specific $CD8^+$ T cells

At a similar time, studies of the $CD8^+$ T-cell response to individual epitopes from EBV latent proteins in patients with IM, using limiting dilution analysis (LDA), were performed (Ref. 50). In these assays, cultures containing different numbers of input lymphocytes are stimulated with antigen in order to drive the expansion of any antigen-specific T cells present. The presence of the expanded populations of antigen-specific T cells is then detected by assaying for their ability to kill target cells expressing the appropriate antigen in cytotoxicity assays. Analysis of results obtained from cultures derived from different numbers of input lymphocytes allows one to derive an estimate of the frequency of T cells specific for a given epitope within the original lymphocyte population. Results suggested that responses to a single epitope from EBV might involve up to 1% of $CD8^+$ T cells. Although this represents a substantial number of $CD8^+$ T cells, the proposed magnitude of these responses clearly differed from that suggested by the repertoire studies.

Enumeration of EBV-specific $CD8^+$ T cells using tetramers

An understanding of the reasons for the discrepancy came with the development of tetramer technology. Synthetic constructs of

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

multimers (tetramers) of major histocompatibility complex (MHC) molecules complexed with a peptide were shown to bind stably to T cells expressing receptors for the appropriate MHC–peptide complex. Labelling such constructs with a fluorescent dye allowed for direct identification of epitope-specific $CD8^+$ T cells in samples of peripheral blood (Ref. 51). Comparison of estimates of T-cell frequencies derived using tetramers and LDA have shown real differences between results obtained with the different methods, with tetramers often detecting an order of magnitude more antigen-specific cells than the LDA assays (Ref. 52). This probably reflects the requirement for cells to proliferate, survive and kill in order to be detected in an LDA; only a proportion of antigen-specific cells might have these capabilities, and others will not be detected by this type of assay.

As importantly, the use of tetramers facilitated the study of responses to a wider range of epitopes. Early functional studies had focused on the EBV latent proteins, whereas reports of responses to EBV lytic cycle proteins were published later (Ref. 53). Tetramers provided the opportunity to study reactivity to epitopes from both the latent and the lytic cycle proteins in a given individual. It became clear that the magnitude of the $CD8^+$ T-cell response varied considerably according to the antigen recognised. Responses to the latent proteins were relatively small in magnitude during primary EBV infection and, in general, responses to individual latent protein epitopes comprised fewer than 2.5% of $CD8^+$ T cells. By contrast, responses to epitopes from the lytic cycle proteins were much larger and could comprise up to 44% of $CD8^+$ T cells (Refs 54, 55). The magnitudes of these latter primary responses were entirely consistent with the results of the TCR repertoire studies and suggest that EBV drives the striking proliferation of specific $CD8^+$ T cells. It has been possible to calculate that, on average, almost a half of the $CD45R0^+CD8^+$ T cells found in patients with IM divide each day (Ref. 56).

In many ways, it is surprising to find such high frequencies of EBV-specific $CD8^+$ T cells within peripheral blood, particularly given the evidence that the site of virus replication and of latent gene expression is largely restricted to tonsillar tissue. A recent study has demonstrated that $CD8^+$ T cells specific for EBV lytic cycle proteins are present within the tonsils with a frequency that is similar to, rather than much greater than, that found in peripheral blood (Ref. 57). The dominance of populations of $CD8^+$ T cells specific for immediate-early and early lytic proteins over those specific for latent proteins in patients with IM has been confirmed in several studies (Refs 54, 55, 58). Although it remains largely unexplained, it has been hypothesised that antigen dose and presentation might influence the magnitude of T-cell responses. Killing of EBV-infected cells by EBV-specific $CD8^+$ T cells would be expected to prevent completion of viral replication within the cell and this in itself would result in immediate-early and some early EBV antigens becoming the predominant viral proteins expressed and hence perhaps stimulating the strongest response. Viral antigens released during lytic infection would probably be taken up and presented efficiently by dendritic cells. However, one might speculate that epitopes from the viral latent proteins could be more likely to be presented directly by the latently infected B cells than indirectly by dendritic cells. Again, it is possible that this will influence the T-cell response.

Functional properties of EBV-specific $CD8^+$ T cells

The expanded populations of EBV-specific $CD8^+$ T cells from patients with IM often express high levels of intracellular perforin and have cytolytic capacity (Ref. 59). They have a highly variable capacity to express interferon (IFN)-γ, at least in ex vivo assays. One study showed that 20–60% of activated $CD8^+$ T cells in patients with IM could respond to the autologous B-lymphoblastoid cell line by expressing IFN-γ (Ref. 60). A second study showed that some epitope-specific cells, identified by tetramer technology, were capable of expressing IFN-γ following stimulation with the relevant peptide in vitro (Ref. 59). Again, it was noted that only a subpopulation of the specific cells (always fewer than 75%) responded. There is no current evidence demonstrating that some of the EBV-reactive $CD8^+$ T cells express only interleukin (IL)-2 or type II cytokines such as IL-4 as an alternative. However, it is quite possible that the cells have expressed high levels of type I cytokines in vivo and are consequently 'functionally exhausted' and perhaps soon to be deleted. Certainly, the antigen-specific $CD8^+$ T cells are highly susceptible to apoptosis. Some of these cells stain with annexin V directly ex vivo, reflecting phosphatidylserine exposure at the cell

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

surface; this is an early sign of apoptosis, and cells die rapidly in vitro (Ref. 59).

Downregulation of primary CD8⁺ T-cell responses

In vivo, the majority of EBV-specific cells die as the primary infection is controlled. This is particularly true of CD8⁺ T cells reactive with the lytic cycle proteins. The time period for the downregulation of the response is variable but can last for several months or even in excess of a year (Refs 54, 55, 58, 59). Over this time, many of the surviving CD8⁺ T cells specific for the lytic cycle proteins can switch from expressing the R0 to the RA isoform of CD45 (Refs 54, 55); the significance of this remains unclear and there is no evidence that this influences the capacity of these cells for cytotoxicity or cytokine expression (Ref. 61). Interestingly, over the same period of time, the frequency of CD8⁺ T cells specific for the latent proteins remains much more stable and can even increase. Although these cells do not usually upregulate CD45RA, an increasing proportion expresses the lymph-node-homing receptor CD62L and the chemokine receptor CCR7 (Ref. 55). One would predict that this would affect the homing capacity of these cells and allow them to access the lymphoid organs where the relevant antigens are likely to be expressed.

Thus, in the long-term, small populations of CD8⁺ T cells specific for both lytic cycle and latent proteins remain within an individual. These cells provide protection during the life-long persistent phase of virus infection.

CD4⁺ T-cell immunity to EBV in patients with IM

Although the overall CD4⁺ T-cell compartment expands little, if at all, in patients with IM, many of the cells express CD38, suggesting activation. Again, there is debate as to whether this is a bystander effect or reflects engagement with cognate antigen. Analysis of the TCR repertoire of CD4⁺ T cells does not support the idea that there is massive antigen-driven clonal expansion. This implies that the primary CD4⁺ T-cell responses to individual EBV epitopes are low in magnitude and/or broad in terms of TCR use (Ref. 48).

Recent studies have provided evidence of a primary CD4⁺ T-cell response to EBV in symptomatic patients. Thus, when peripheral blood mononuclear cells from patients with IM were cultured with a preparation of EBV antigens, a proportion of the CD4⁺ T cells responded by expressing IFN-γ (Ref. 62). Using this experimental approach it was deduced that, in a population of patients with IM, a mean of 1.4% circulating CD4⁺ T cells were able to recognise the EBV proteins, with up to 5.2% of CD4⁺ T cells being specific for these antigens in some individuals. The EBV-specific CD4⁺ T cells expressed CD45R0 and CD38, confirming the idea that they were activated, antigen-experienced cells. The primary response appeared to be characterised by a primary burst, as responses at four months were much lower, with a mean of 0.22% of CD4⁺ T cells being reactive with EBV proteins. Importantly, a further study demonstrated specificity of responses and showed that CD4⁺ T-cell reactivity to the lytic cycle proteins BZLF1 and BMLF1 and the latent protein EBNA1 was found in the majority of individuals with IM, whereas responses to the other latent protein studied, EBNA3A, were less commonly found (Ref. 63). In this work, the median size of the responses to the individual proteins ranged from 0.16–0.31% of CD4⁺ T cells during primary infection. Responses to all proteins fell with time, again consistent with the idea of a primary burst in the CD4⁺ T-cell response in patients with IM.

The studies have shown that the CD4⁺ T cells are capable of expressing cytokines, including IFN-γ, directly ex vivo. Little else is known about the functional capacity of EBV-specific CD4⁺ T-cell responses during primary EBV infection or about their overall role in controlling infection. Interesting work performed using EBV-specific T-cell clones derived from the blood of healthy individuals who are EBV seropositive shows that these are sometimes capable of recognising and even killing the autologous EBV-transformed B-cell line (Refs 64, 65, 66, 67, 68). This raises the possibility that EBV-specific CD4⁺ T cells might play a direct role in killing virus-infected cells as well as in expressing cytokines and providing help for CD8⁺ T cells and B cells.

Disease associated with primary infection

Worldwide, EBV infection usually occurs within the first few years of life and does not cause clinically important illness. However, if primary infection is delayed until adolescence or beyond, it is associated with the clinical syndrome of IM in approximately 25–50% of cases (Table 2) (Ref. 69). The incubation period is thought to last 4–7 weeks and is usually followed by the development of pharyngitis accompanied by

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

fevers, lymphadenopathy and splenomegaly. Fatigue and mild depression are commonly reported. Most patients have biochemical evidence of hepatitis, although jaundice occurs in fewer than 10% of cases. Palatal petechiae and, more rarely, a morbilliform rash may also accompany disease. Occasionally patients suffer from cardiac, respiratory or renal complications. Central nervous system involvement is well recognised, with the development of meningitis, encephalitis, transverse myelitis and mono- or polyneuritis all being described. Interestingly, in these cases, the more typical features of pharyngitis and lymphadenopathy are often absent. Although a slightly low platelet count is commonly seen, severe thrombocytopenia resulting in haemorrhage is most unusual. IM is only very rarely fatal; deaths have resulted from splenic rupture, respiratory tract obstruction, fulminant hepatitis and haemophagocytosis, as well as from neurological complications, particularly encephalitis.

In the majority of individuals, the disease is self-limiting within 3–6 weeks and no specific treatment is required. High-dose acyclovir may decrease the load of replicating virus within the oropharynx but does not influence the clinical course of the disease (Ref. 70). Prednisolone is indicated for patients with severe disease, particularly those suffering from neurological complications or at risk of splenic rupture or respiratory obstruction (Ref. 71). Amoxycillin should not be prescribed as this drug causes a rash in 90% of patients with IM.

The clinical illness of IM could reflect three components of the disease: (1) cell damage that is mediated by virus replicating in the oropharynx; (2) B-cell lymphoproliferation and cytokine expression that is dependent on the virus establishing growth-transforming latent infection within B cells; and (3) immunopathology secondary to the very striking immune response. Of these components, it is likely that the majority of the systemic features of IM reflect immunopathology. In support of this, there is evidence for high levels of circulating cytokines in patients, including tumour necrosis factor (TNF)-α and IFN-γ (Refs 72, 73), reflecting a potent immune response. The hypothesis would also be consistent with the failure of the disease to respond to attempts to control viral load using acyclovir, and its sensitivity to the use of steroid to suppress the immune response.

Table 2. Clinical features of infectious mononucleosis

Organ	Clinical feature
Systemic	Fevers Malaise
Oropharynx	Pharyngitis; sometimes severe and occasionally complicated by airway obstruction Palatal petechiae
Lymphoid system	Lymphadenopathy Splenomegaly; occasionally complicated by infarction or splenic rupture
Liver	Hepatitis; usually mild, with elevated transaminases Jaundice in 5–10% of cases
Skin	Transient morbilliform rash Drug rash induced by ampicillin
Heart	Electrocardiogram abnormalities with T wave changes Cardiac conduction defects Myocarditis Pericarditis
Lungs	Pneumonitis
Kidney	Microscopic haematuria Interstitial nephritis Glomerulonephritis
Central nervous system	Meningitis Encephalitis Mono- or polyneuritis Transverse myelitis
Haematological	Thrombocytopenia; usually mild Haemolytic anaemia Neutropenia
Secondary infection	Beta-haemolytic *Streptococcus*

Clinical practice reveals that IM is very variable in its severity, with the minority of patients suffering severe and even life-threatening disease, and the majority having symptoms that temporarily prevent them from continuing with normal activities. Some individuals are less severely affected. Furthermore, over 50% of adults and most children suffer no important symptoms and seroconvert silently. Debate continues as to

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

why this should be so. Differences in initial viral load might be one factor; children, for example, might receive a smaller dose of infecting virus from parents and siblings than adolescents who acquire the virus from kissing. Variation in the immune response to the virus might also be important. For instance, there might be differences in cytokine profiles of responding T cells between young children and adults and even between different adults. There might also be important differences in the strength of the primary immune response. In this respect, one interesting study has shown that adults who undergo silent seroconversion do not show evidence of $CD8^+$ T-cell expansions, whereas those with symptoms do show evidence (Ref. 74). Importantly, virus load was high and similar in the two groups analysed in this study and, although the conclusions are based on very limited numbers, this important work raises the question as to why different individuals might mount immune responses of different strengths in the face of similar challenge. One idea that is relevant in this respect is that of 'heterologous immunity'. Prior exposure to pathogens will generate a repertoire of 'primed' T cells. Some of these might be cross-reactive with epitopes from EBV and be capable of very rapid expansion and acquisition of effector function (features normally associated with a secondary immune response) during primary EBV infection. Thus an individual's history of infection might be an important factor in determining the strength of an immune response to a new pathogen (Ref. 75). Further detailed studies are needed to determine the virological and immunological correlates for disease severity in patients with IM.

Very rarely, patients, particularly children, suffer from severe and recurrent or persistent symptoms (Refs 76, 77). These can be associated with a persistently high EBV load and a failure in the normal maturation of the antibody response. Importantly, EBV infection within T cells or natural killer (NK) cells as well as B cells is commonly found in these patients. The condition is defined as chronic active EBV infection, is most common in Japan, and carries a very poor prognosis with a mortality that approaches 50%. Patients usually die of lymphoma, haemophagocytic syndrome or fulminant hepatitis.

Primary EBV infection also represents a serious threat in two other groups of individuals. First, as discussed above, males with X-linked lymphoproliferative syndrome have defects within their NK- and T-cell signalling pathways and usually develop a fatal form of IM following primary EBV infection (Ref. 41). Second, individuals who develop primary infection when they are immunosuppressed are often unable to control the virus infection and develop B-cell lymphoproliferative disease. These patients may present with IM-like symptoms or with extra-nodal B-cell lymphomas, sometimes affecting the gut or the brain (Refs 78, 79).

Future directions: the development of a vaccine for IM

The morbidity associated with primary EBV infection makes a case for the development of a vaccine for prevention of IM; such a vaccine would be targeted to EBV-seronegative adolescents and to EBV-seronegative children being considered for transplantation and immunosuppression. Although the ultimate aim for such a vaccine would be to prevent primary infection, the preparation could also be effective in preventing the development of malignancies associated with persistent EBV infection. However, a vaccine that was capable of significantly reducing EBV load during primary infection without actually preventing the establishment of persistent infection might also be effective in reducing the symptoms of IM and could therefore have important benefits. There are theoretical hazards associated with this approach, and careful immunological studies should accompany clinical trials. First, it is possible that prior vaccination might result in individuals mounting a 'secondary' cellular immune response following their first encounter with EBV itself; such a 'secondary' response might be even greater than the primary response usually elicited by EBV and result in more immunopathology. Second, the risk of malignant disease associated with persistent infection would be likely to remain. Vaccinated individuals might well mount a different immune response from nonvaccinated individuals after virus challenge. Whether this immune response would be as effective in protecting individuals against EBV-associated malignant disease in the long-term is unknown.

The observation that neutralising antibodies are specific for gp350 and can block virus attachment to cells has stimulated interest in the possible efficacy of a vaccine preparation based on this glycoprotein. Vaccination of experimental

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

animals with purified gp350 will stimulate production of specific antibodies; such antibodies are able to neutralise EBV infection in vitro (Refs 80, 81). A more recent study has shown that immunisation of mice with a plasmid vector containing the gene for gp350 induced IgG1 antibodies that were capable of mediating antibody-dependent cellular cytotoxicity against cells expressing gp350 (Ref. 82). The DNA vector stimulated both a cell-mediated and a humoral response in these mice. Cytotoxic T lymphocyte responses against gp350 have also been elicited in mice by immunising with a peptide representing an epitope from gp350; such lymphocytes were capable of protecting against challenge with recombinant vaccinia virus expressing gp350 (Ref. 83). T-cell responses to gp350 have been documented in humans; $CD8^+$ T cells specific for this glycoprotein have been found in individuals with primary EBV infection (Ref. 83) and $CD4^+$ T-cell responses have been demonstrated in two studies, both in the context of persistent infection (Refs 62, 84). However, more work is needed to investigate the relative immunodominance of gp350 as an antigen for both $CD8^+$ and $CD4^+$ T-cell immunity. As a candidate vaccine, it would be advantageous if gp350 stimulated strong T-cell responses in the majority of individuals irrespective of their major histocompatibility complex HLA types. Nevertheless, the animal studies and the data available from human studies provide some support for the development of a gp350-based vaccine. Early clinical trials of this type of vaccine have been undertaken in humans to test safety; larger studies are now needed to confirm efficacy.

Concluding remarks

Primary EBV infection is a potentially dangerous event that involves not only virus replication but also growth transformation of B cells. Both virus and host have strategies that serve to prevent the possible fatal consequences of EBV infection. The virus is capable of tightly regulating expression of its genes, while the host is able to mount an aggressive immune response; indeed the $CD8^+$ T-cell component of this is greater than has been reported in any other primary infection of humans.

A picture is emerging in which lytic and growth-transforming forms of EBV infection are largely restricted in location. The viral proteins expressed in the tonsillar region stimulate a vigorous $CD8^+$ T-cell response and these cytotoxic cells play an important role in controlling virus. The functional competence of these $CD8^+$ T cells might well depend on an associated $CD4^+$ T-cell response. Whereas infected B cells do emerge into the peripheral circulation, recent work suggests that viral proteins are not generally expressed within these cell, even during primary infection. Such B cells are therefore not immediately harmful but might act as a reservoir for virus infection. When these latently infected B cells recirculate to the tonsils, re-institution of replicative infection might occur.

As viral replication is controlled within the oropharynx, the antigen load falls and there is a fall in the frequency of $CD8^+$ T cells specific for lytic cycle proteins. Control of viral infection in the tonsils is also accompanied by a fall in the numbers of EBV-infected B cells that emerge into the periphery. Consistent with this type of model, experimental work suggests that the decay in the EBV-specific cytotoxic T-cell response is similar to the decay in the numbers of EBV-infected B cells in the periphery. The host–virus balance tends towards an equilibrium that allows the virus to achieve a form of harmless, persistent infection in the vast majority of individuals. It is envisaged that there are low levels of ongoing virus replication and possibly growth-transforming infection in the tonsillar/lymphoid regions, controlled by the residual populations of EBV-specific T cells. In association, small numbers of latently infected B cells circulate.

Although understanding of how this host–virus relationship is achieved has increased enormously over the past decade, large numbers of questions remain unanswered. Almost nothing is known about the very early events after primary infection, before the development of clinical symptoms. Details of the biology of primary virus infection are still lacking. Understanding of important components of the immune response, including the innate response and the $CD4^+$ T-cell response remain very incomplete. Knowledge about the correlates, either virological or immunological, for the severity of clinical disease during primary infection is lacking. Some of these issues will be very difficult to address, as early or asymptomatic primary infection is not usually identifiable. All require clinical samples taken from patients. The importance of the research effort is, however, already being seen in the

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

development of new therapies for the virus, particularly in immunocompromised individuals where the normally successful host–virus balance is altered.

Acknowledgements and funding

Margaret Callan is funded by a Senior Clinical Fellowship from the Medical Research Council. Eleni-Kyriaki Vetsika is also funded by the Medical Research Council. We thank the anonymous peer referees for their comments.

References

1 Epstein, M.A., Achong, B.G. and Barr, Y.M. (1964) Virus Particles in Cultured Lymphoblasts from Burkitt's Lymphoma. Lancet 15, 702-703, PubMed: 14107961
2 Henle, G., Henle, W. and Diehl, V. (1968) Relation of Burkitt's tumor-associated herpes-ytpe virus to infectious mononucleosis. Proc Natl Acad Sci U S A 59, 94-101, PubMed: 5242134
3 Niederman, J.C. et al. (1968) Infectious mononucleosis. J Am Med Assoc 203, 139-143
4 Henle, G., Henle, W. and Horwitz, C.A. (1974) Antibodies to Epstein-Barr virus associated nuclear antigen in infectious mononucleosis. J Inf Dis 130, 231-239
5 Henle, G. et al. (1969) Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. J Natl Cancer Inst 43, 1147-1157, PubMed: 5353242
6 Fleisher, G. et al. (1979) Primary infection with Epstein-Barr virus in infants in the United States: clinical and serologic observations. J Infect Dis 139, 553-558, PubMed: 220340
7 Fleisher, G.R. et al. (1981) Intrafamilial transmission of Epstein-Barr virus infections. J Pediatr 98, 16-19, PubMed: 7452392
8 Hallee, T.J. et al. (1974) Infectious mononucleosis at the United States Military Academy. A prospective study of a single class over four years. Yale J Biol Med 47, 182-195, PubMed: 4374836
9 Sawyer, R.N. et al. (1971) Prospective studies of a group of Yale University freshmen. I. Occurrence of infectious mononucleosis. J Infect Dis 123, 263-270, PubMed: 4329526
10 Lopes, V., Young, L.S. and Murray, P.G. (2003) Epstein-Barr virus-associated cancers: aetiology and treatment. Herpes 10, 78-82, PubMed: 14759340
11 Thorley-Lawson, D.A. and Gross, A. (2004) Persistence of the Epstein-Barr virus and the origins of associated lymphomas. N Engl J Med 350, 1328-1337, PubMed: 15044644
12 Hopwood, P. and Crawford, D.H. (2000) The role of EBV in post-transplant malignancies: a review. J Clin Pathol 53, 248-254, PubMed: 10823119
13 Baer, R. et al. (1984) DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature 310, 207-211, PubMed: 6087149
14 Kieff, E. and Rickinson, A.B. (2001) Epstein-Barr virus and its replication. In Virology (Knipe, D.M. and Howley, P.M., eds), pp. 2511-2574, Lippincott Williams and Wilkins, New York
15 Murray, P.G. and Young, L.S. (2001) Epstein-Barr virus infection: basis of malignancy and potential for therapy. Expert Rev Mol Med 2001, 1-20, PubMed: 14585152
16 Hammerschmidt, W. and Sugden, B. (1989) Genetic analysis of immortalizing functions of Epstein-Barr virus in human B lymphocytes. Nature 340, 393-397, PubMed: 2547164
17 Hickabottom, M. et al. (2002) Two nonconsensus sites in the Epstein-Barr virus oncoprotein EBNA3A cooperate to bind the co-repressor carboxyl-terminal-binding protein (CtBP). J Biol Chem 277, 47197-47204, PubMed: 12372828
18 Parker, G.A. et al. (1996) Epstein-Barr virus nuclear antigen (EBNA)3C is an immortalizing oncoprotein with similar properties to adenovirus E1A and papillomavirus E7. Oncogene 13, 2541-2549, PubMed: 9000128
19 Young, L. et al. (1989) Expression of Epstein-Barr virus transformation-associated genes in tissues of patients with EBV lymphoproliferative disease. N Engl J Med 321, 1080-1085, PubMed: 2552313
20 Babcock, G.J. et al. (1998) EBV persistence in memory B cells in vivo. Immunity 9, 395-404, PubMed: 9768759
21 Joseph, A.M., Babcock, G.J. and Thorley-Lawson, D.A. (2000) Cells expressing the Epstein-Barr virus growth program are present in and restricted to the naive B-cell subset of healthy tonsils. J Virol 74, 9964-9971, PubMed: 11024124
22 Babcock, G.J. and Thorley-Lawson, D.A. (2000) Tonsillar memory B cells, latently infected with Epstein-Barr virus, express the restricted pattern of latent genes previously found only in Epstein-Barr virus-associated tumors. Proc Natl Acad Sci U S A 97, 12250-12255, PubMed: 11035774
23 Babcock, G.J., Hochberg, D. and Thorley-Lawson, A.D. (2000) The expression pattern of Epstein-Barr virus latent genes in vivo is dependent upon the differentiation stage of the infected B cell. Immunity 13, 497-506, PubMed:

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

11070168
24 Thorley-Lawson, D.A. (2001) Epstein-Barr virus: exploiting the immune system. Nat Rev Immunol 1, 75-82, PubMed: 11905817
25 Niedobitek, G. et al. (2000) Epstein-Barr virus (EBV) in infectious mononucleosis: detection of the virus in tonsillar B lymphocytes but not in desquamated oropharyngeal epithelial cells. Mol Pathol 53, 37-42, PubMed: 10884920
26 Anagnostopoulos, I. et al. (1995) Morphology, immunophenotype, and distribution of latently and/or productively Epstein-Barr virus-infected cells in acute infectious mononucleosis: implications for the interindividual infection route of Epstein-Barr virus. Blood 85, 744-750, PubMed: 7530505
27 Izumi, K.M. and Kieff, E.D. (1997) The Epstein-Barr virus oncogene product latent membrane protein 1 engages the tumor necrosis factor receptor-associated death domain protein to mediate B lymphocyte growth transformation and activate NF-kappaB. Proc Natl Acad Sci U S A 94, 12592-12597, PubMed: 9356494
28 Gires, O. et al. (1997) Latent membrane protein 1 of Epstein-Barr virus mimics a constitutively active receptor molecule. Embo J 16, 6131-6140, PubMed: 9359753
29 Uchida, J. et al. (1999) Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B lymphocyte responses. Science 286, 300-303, PubMed: 10514374
30 Caldwell, R.G. et al. (1998) Epstein-Barr virus LMP2A drives B cell development and survival in the absence of normal B cell receptor signals. Immunity 9, 405-411, PubMed: 9768760
31 Hochberg, D. et al. (2004) Demonstration of the Burkitt's lymphoma Epstein-Barr virus phenotype in dividing latently infected memory cells in vivo. Proc Natl Acad Sci U S A 101, 239-244, PubMed: 14688409
32 Kurth, J. et al. (2000) EBV-infected B cells in infectious mononucleosis: viral strategies for spreading in the B cell compartment and establishing latency. Immunity 13, 485-495, PubMed: 11070167
33 Kurth, J. et al. (2003) Epstein-Barr virus-infected B cells expanding in germinal centers of infectious mononucleosis patients do not participate in the germinal center reaction. Proc Natl Acad Sci U S A 100, 4730-4735, PubMed: 12665622
34 Kuppers, R. (2003) B cells under influence: transformation of B cells by Epstein-Barr virus. Nat Rev Immunol 3, 801-812, PubMed: 14523386
35 Crawford, D.H. et al. (1978) Epstein-Barr (EB) virus genome-containing, EB nuclear antigen-negative B-lymphocyte populations in blood in acute infectious mononucleosis. J Gen Virol 38, 449-460, PubMed: 204732
36 Robinson, J., Smith, D. and Niederman, J. (1980) Mitotic EBNA-positive lymphocytes in peripheral blood during infectious mononucleosis. Nature 287, 334-335, PubMed: 6252472
37 Tierney, R.J. et al. (1994) Epstein-Barr virus latency in blood mononuclear cells: analysis of viral gene transcription during primary infection and in the carrier state. J Virol 68, 7374-7385, PubMed: 7933121
38 Prang, N.S. et al. (1997) Lytic replication of Epstein-Barr virus in the peripheral blood: analysis of viral gene expression in B lymphocytes during infectious mononucleosis and in the normal carrier state. Blood 89, 1665-1677, PubMed: 9057649
39 Hochberg, D. et al. (2004) Acute infection with Epstein-Barr virus targets and overwhelms the peripheral memory B-cell compartment with resting, latently infected cells. J Virol 78, 5194-5204, PubMed: 15113901
40 Parolini, S. et al. (2000) X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. J Exp Med 192, 337-346, PubMed: 10934222
41 Sayos, J. et al. (1998) The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. Nature 395, 462-469, PubMed: 9774102
42 Benoit, L. et al. (2000) Defective NK cell activation in X-linked lymphoproliferative disease. J Immunol 165, 3549-3553, PubMed: 11034354
43 Nakajima, H. et al. (2000) Patients with X-linked lymphoproliferative disease have a defect in 2B4 receptor-mediated NK cell cytotoxicity. Eur J Immunol 30, 3309-3318, PubMed: 11093147
44 Sharifi, R. et al. (2004) SAP mediates specific cytotoxic T-cell functions in X-linked lymphoproliferative disease. Blood 103, 3821-3827, PubMed: 14726378
45 Garzelli, C. et al. (1984) Epstein-Barr virus-transformed lymphocytes produce monoclonal

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

autoantibodies that react with antigens in multiple organs. J Virol 52, 722-725, PubMed: 6092698
46 Soots, M.L. (1969) Specificity of the Paul-Bunnell test. Br Med J 3, 415, PubMed: 5819596
47 Callan, M.F. et al. (1996) Large clonal expansions of CD8+ T cells in acute infectious mononucleosis. Nat Med 2, 906-911, PubMed: 8705861
48 Maini, M.K. et al. (2000) Clonal expansions in acute EBV infection are detectable in the CD8 and not the CD4 subset and persist with a variable CD45 phenotype. J Immunol 165, 5729-5737, PubMed: 11067931
49 Posnett, D.N. et al. (1994) Clonal populations of T cells in normal elderly humans: the T cell equivalent to "benign monoclonal gammapathy". J Exp Med 179, 609-618, PubMed: 8294871
50 Steven, N.M. et al. (1996) Epitope focusing in the primary cytotoxic T cell response to Epstein-Barr virus and its relationship to T cell memory. J Exp Med 184, 1801-1813, PubMed: 8920868
51 Altman, J.D. et al. (1996) Phenotypic analysis of antigen-specific T lymphocytes. Science 274, 94-96, PubMed: 8810254
52 Tan, L.C. et al. (1999) A re-evaluation of the frequency of CD8+ T cells specific for EBV in healthy virus carriers. J Immunol 162, 1827-1835, PubMed: 9973448
53 Bogedain, C. et al. (1995) Specific cytotoxic T lymphocytes recognize the immediate-early transactivator Zta of Epstein-Barr virus. J Virol 69, 4872-4879, PubMed: 7609055
54 Callan, M.F. et al. (1998) Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstein-Barr virus In vivo. J Exp Med 187, 1395-1402, PubMed: 9565632
55 Hislop, A.D. et al. (2002) Epitope-specific evolution of human CD8(+) T cell responses from primary to persistent phases of Epstein-Barr virus infection. J Exp Med 195, 893-905, PubMed: 11927633
56 Macallan, D.C. et al. (2003) Rapid turnover of T cells in acute infectious mononucleosis. Eur J Immunol 33, 2655-2665, PubMed: 14515249
57 Soares, M.V. et al. (2004) Integration of apoptosis and telomere erosion in virus-specific CD8+ T cells from blood and tonsils during primary infection. Blood 103, 162-167, PubMed: 12969961
58 Catalina, M.D. et al. (2001) Differential evolution and stability of epitope-specific CD8(+) T cell responses in EBV infection. J Immunol 167, 4450-4457, PubMed: 11591771
59 Callan, M.F. et al. (2000) CD8(+) T-cell selection, function, and death in the primary immune response in vivo. J Clin Invest 106, 1251-1261, PubMed: 11086026
60 Hoshino, Y. et al. (1999) Antigen-driven expansion and contraction of CD8+-activated T cells in primary EBV infection. J Immunol 163, 5735-5740, PubMed: 10553106
61 Hislop, A.D. et al. (2001) EBV-specific CD8+ T cell memory: relationships between epitope specificity, cell phenotype, and immediate effector function. J Immunol 167, 2019-2029, PubMed: 11489984
62 Amyes, E. et al. (2003) Characterization of the CD4+ T cell response to Epstein-Barr virus during primary and persistent infection. J Exp Med 198, 903-911, PubMed: 12975456
63 Precopio, M.L. et al. (2003) Differential kinetics and specificity of EBV-specific CD4+ and CD8+ T cells during primary infection. J Immunol 170, 2590-2598, PubMed: 12594286
64 Khanna, R. et al. (1997) Class I processing-defective Burkitt's lymphoma cells are recognized efficiently by CD4+ EBV-specific CTLs. J Immunol 158, 3619-3625, PubMed: 9103423
65 Munz, C. et al. (2000) Human CD4(+) T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. J Exp Med 191, 1649-1660, PubMed: 10811859
66 Paludan, C. et al. (2002) Epstein-Barr nuclear antigen 1-specific CD4(+) Th1 cells kill Burkitt's lymphoma cells. J Immunol 169, 1593-1603, PubMed: 12133989
67 Nikiforow, S. et al. (2003) Cytolytic CD4(+)-T-cell clones reactive to EBNA1 inhibit Epstein-Barr virus-induced B-cell proliferation. J Virol 77, 12088-12104, PubMed: 14581546
68 Landais, E. et al. (2004) Direct killing of Epstein-Barr virus (EBV)-infected B cells by CD4 T cells directed against the EBV lytic protein BHRF1. Blood 103, 1408-1416, PubMed: 14563644
69 Schooley, R.T. (2000) Epstein-Barr virus (infectious mononucleosis). In Principles and Practice of Infectious Diseases (5th edn) (Mandell, G.L., Bennett, J.E. and Doline, R., eds), pp. 1599-1613, Churchill Livingstone, Philadelphia, USA
70 Andersson, J. et al. (1986) Effect of acyclovir on infectious mononucleosis: a double-blind, placebo-controlled study. J Infect Dis 153, 283-290, PubMed: 3003206

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

71 McGowan, J.E., Jr. et al. (1992) Guidelines for the use of systemic glucocorticosteroids in the management of selected infections. Working Group on Steroid Use, Antimicrobial Agents Committee, Infectious Diseases Society of America. J Infect Dis 165, 1-13, PubMed: 1727879
72 Kaminska, T. et al. (1996) Interferon and tumor necrosis factor production by peripheral blood leukocytes of patients with infectious mononucleosis. Arch Immunol Ther Exp (Warsz) 44, 353-357, PubMed: 9017151
73 Wright-Browne, V. et al. (1998) Serum cytokine levels in infectious mononucleosis at diagnosis and convalescence. Leuk Lymphoma 30, 583-589, PubMed: 9711920
74 Silins, S.L. et al. (2001) Asymptomatic primary Epstein-Barr virus infection occurs in the absence of blood T-cell repertoire perturbations despite high levels of systemic viral load. Blood 98, 3739-3744, PubMed: 11739180
75 Brehm, M.A., Selin, L.K. and Welsh, R.M. (2004) CD8 T cell responses to viral infections in sequence. Cell Microbiol 6, 411-421, PubMed: 15056212
76 Straus, S.E. (1988) The chronic mononucleosis syndrome. J Infect Dis 157, 405-412, PubMed: 2830340
77 Kimura, H. et al. (2001) Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. Blood 98, 280-286, PubMed: 11435294
78 Haque, T. et al. (1996) Transmission of donor Epstein-Barr virus (EBV) in transplanted organs causes lymphoproliferative disease in EBV-seronegative recipients. J Gen Virol 77 (Pt 6), 1169-1172, PubMed: 8683203
79 Ho, M. et al. (1985) Epstein-Barr virus infections and DNA hybridization studies in posttransplantation lymphoma and lymphoproliferative lesions: the role of primary infection. J Infect Dis 152, 876-886, PubMed: 2995512
80 Thorley-Lawson, D.A. and Poodry, C.A. (1982) Identification and isolation of the main component (gp350-gp220) of Epstein-Barr virus responsible for generating neutralizing antibodies in vivo. J Virol 43, 730-736, PubMed: 6287039
81 Jackman, W.T. et al. (1999) Expression of Epstein-Barr virus gp350 as a single chain glycoprotein for an EBV subunit vaccine. Vaccine 17, 660-668, PubMed: 10067671
82 Jung, S. et al. (2001) DNA-mediated immunization of glycoprotein 350 of Epstein-Barr virus induces the effective humoral and cellular immune responses against the antigen. Mol Cells 12, 41-49, PubMed: 11561729
83 Khanna, R., Sherritt, M. and Burrows, S.R. (1999) EBV structural antigens, gp350 and gp85, as targets for ex vivo virus-specific CTL during acute infectious mononucleosis: potential use of gp350/gp85 CTL epitopes for vaccine design. J Immunol 162, 3063-3069, PubMed: 10072559
84 Wallace, L.E. et al. (1991) Identification of two T-cell epitopes on the candidate Epstein-Barr virus vaccine glycoprotein gp340 recognized by CD4+ T-cell clones. J Virol 65, 3821-3828, PubMed: 1710291

Further reading, resources and contacts

Information sheet on Epstein–Barr virus and infectious mononucleosis by the National Center for Infectious Diseases:

http://www.cdc.gov/ncidod/diseases/ebv.htm

Kissing the Epstein–Barr virus goodbye (published by the Australian Academy of Science):

http://www.science.org.au/nova/026/026key.htm

Mononucleosis (published by the Mayo clinic):

http://www.mayoclinic.com/invoke.cfm?id=DS00352

Epstein–Barr virus (from Virology Down Under):

http://www.uq.edu.au/vdu/EBV.htm

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press

Features associated with this article

Figures

Figure 1. A model for Epstein–Barr virus (EBV) infection and persistence.

Tables

Table 1. Patterns of Epstein–Barr virus (EBV) gene expression in forms of latent infection identified in different EBV-associated malignancies.
Table 2. Clinical features of infectious mononucleosis.

Citation details for this article

Eleni-Kyriaki Vetsika and Margaret Callan (2004) Infectious mononucleosis and Epstein–Barr virus. Expert Rev. Mol. Med. Vol. 6, Issue 23, 5 November, DOI: 10.1017/S1462399404008440

Accession information: DOI: 10.1017/S1462399404008440; Vol. 6; Issue 23; 5 November 2004
©2004 Cambridge University Press

https://doi.org/10.1017/S1462399404008440 Published online by Cambridge University Press