Kaposi sarcoma herpesvirus (KSHV), a human gammaherpesvirus, is the etiological agent for the endothelial-derived Kaposi sarcoma (KS) and also for certain lymphoproliferative disorders. In these lymphoproliferations, the KSHV-infected cells carry the stigmata of B lymphocytes, with plasmablastic features. The *JCI* has published three manuscripts addressing key questions related to B cell infection and viral latent expression in B cells. Myoung and Ganem show that CD4+ lymphocytes suppress KSHV replication, promoting latency in B cells; Hassman and colleagues show that KSHV infection drives plasmablast differentiation in a subset of IgM+ λ light chain–expressing cells; and Ballon and colleagues describe the in vivo transdifferentiation of B lymphocytes by KSHV-encoded viral FLICE-inhibitory protein (vFLIP).

Two lymphotropic human herpesviruses are linked to lymphoma development: EBV and Kaposi sarcoma herpesvirus (KSHV). The mechanisms by which EBV infects B lymphocytes and induces their differentiation and proliferation are reasonably well understood (1). In vitro, EBV infection of human primary B cells causes the establishment of latent infection in a fraction of cells exposed to virus, cellular transformation, and the outgrowth of indefinitely proliferating B lymphoblastoid cell lines. In contrast, the lack of B cell systems available for the study of KSHV in vitro and in vivo has hampered our understanding of the natural life cycle of KSHV in B cells and of KSHV-induced B cell lymphoproliferations. The 

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**Unraveling virus-induced lymphomagenesis**

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recognition and might trigger an exocytosis event in the effector T cells, releasing factors to the KSHV-infected B lymphocytes. These in vitro findings contrast with what we have learned about primary EBV infection (10): the current paradigm is that lack of functional T lymphocytes, for example, induced by iatrogenic or acquired immunosuppression, leads to the in vivo outgrowth of latent infected B lymphocytes and subsequent EBV-driven lymphoproliferations such as posttransplant lymphoproliferative disease. Myoung and Ganem propose that T lymphocyte activation is necessary to block KSHV lytic reactivation in B lymphocytes, promoting latent infection (Figure 1).

It is unclear whether B lymphocytes display de novo entry into the lytic cycle (i.e., the lytic cycle being the default pathway) or whether high level spontaneous reactivation occurs from latency. It will be of significant interest to know whether these intriguing findings occur in vivo and whether this suppression of lytic reactivation is restricted to primary infection within the tonsillar microenvironment.

Prior to the introduction of effective anti-HIV treatment, it was noted that nearly 50% of those who acquired KSHV after HIV infection went on to develop Kaposi sarcoma (KS). Thus, being exposed to KSHV in the setting of a damaged T lymphocyte immune response would result in a higher KSHV viral load set point and an increased risk of developing KS. The findings by Myoung and Ganem could help explain this observation: a diminished T lymphocyte response upon primary KSHV infection would result in enhanced spontaneous B lymphocyte reactivation and a higher viral load.

KSHV and plasma cell disorders

B cell neoplasia associated with KSHV infection includes the monoclonal primary effusion lymphoma (PEL) (11) and a plasmablastic variant of multicentric Castleman disease (MCD) (12), a polyclonal neoplasm. In both these tumors, KSHV infection is associated with preterminally differentiated plasma cells with either cytoplasmic or surface immunoglobulins. Mature B cells exhibit allelic exclusion in which only a single class of Ig heavy chain and a single class of light chain, \(\kappa\) or \(\lambda\), is expressed. Light chain restriction in a B cell population is usually considered proof of monoclonality and therefore cancer. However, KSHV-infected plasmablasts in MCD are an exception, in which polyclonal expansion of IgM\(\lambda\)-expressing KSHV-infected plasmablasts is the hallmark (refs. 13–15 and Figure 2).
This is a curiosity, as almost all other lymphoproliferative disorders demonstrate both κ and λ light chain restriction, and no functional difference between these light chains is identified. This observation suggests that KSHV either preferentially infects λ light chain–expressing lymphocytes, that λ light chain cells provide a survival and/or proliferation advantage to KSHV-infected B cells, or that KSHV preferentially steers expansion of λ-expressing cells.

The Kedes group investigated whether KSHV preferentially infects plasmablasts or infects a less differentiated cell and actively drives them toward a plasmablast phenotype (3). In a series of elegant experiments, Hassman et al. employed multispectral imaging flow cytometry, which combines the high-throughput power of flow cytometry with the morphological and subcellular spatial detail of multicolor fluorescent imaging to identify and characterize KSHV-infected cells. First they demonstrated that within ex vivo suspensions of human tonsillar cells, KSHV infection (as measured by cells expressing the latency-associated nuclear antigen [LANA]) preferentially occurred in B cells. Even though both light chain subsets were present in the tonsillar cultures in comparable proportions, LANA-positive cells were almost exclusively observed in the λ subset. They show further that KSHV infection drives the proliferation of IgMκ tonsillar B cells. In addition, infected cells acquired phenotypic changes mimicking MCD plasmablasts, including the blas imaging flow cytometry, which combines the high-throughput power of flow cytometry with the morphological and subcellular spatial detail of multicolor fluorescent imaging to identify and characterize KSHV-infected cells. First they demonstrated that within ex vivo suspensions of human tonsillar cells, KSHV infection (as measured by cells expressing the latency-associated nuclear antigen [LANA]) preferentially occurred in B cells. Even though both light chain subsets were present in the tonsillar cultures in comparable proportions, LANA-positive cells were almost exclusively observed in the λ subset. They show further that KSHV infection drives the proliferation of IgMκ tonsillar B cells. In addition, infected cells acquired phenotypic changes mimicking MCD plasmablasts, including the blasting morphology, IgM expression, and high levels of IL-6 receptor expression.

Hassman et al. raise an intriguing, but plausible possibility: rather than targeting naive B cells, KSHV preferentially infects and drives the proliferation of IgMκ memory B cells. Such cells are present in tonsils and spleen and become IgMκ, Ki67+, and CD27+ during plasmablast differentiation (16). Such differentiation might be triggered by KSHV-induced NF-κB activation (see below), and such a phenotype is compatible with the plasmablasts present in their infected tonsillar cultures and in MCD.

**NF-κB and B cell plasticity**

Similar to other herpesviruses, only a fraction of KSHV open reading frames are expressed during latency, with the majority being expressed when the virus is triggered to enter the lytic program, resulting in cells exuding infectious virions. Among these latent viral proteins is the viral FADD-like IL-1β-converting enzyme (FLICE/caspase 8) inhibitory protein (vFLIP). Whereas cellular FLIP proteins associate with FADD/DISC, preventing caspase-8–induced apoptosis, vFLIP in KSHV-infected cells, including PEL cells, is mainly associated with the IkB kinase (IKK) complex, leading to IkBα degradation, followed by release of NF-κB (17, 18). Activation of NF-κB is a mechanism exploited by lymphomagenic viruses to prolong cellular survival and to induce proliferation. EBV and human T cell leukemia virus 1 (HTLV-1) encode viral oncoproteins, LMP1 and Tax, constitutively activating NF-κB (19, 20).

The Cesarman group generated inducible vFLIP knockin mice, targeting vFLIP to different stages of B cell proliferation (4). The activation of transgene expression was achieved by crossing the vFLIP knockin mice with mice expressing cre recombinase under the control of either the CD19 or Cγ1 promoter, resulting in vFLIP expression in all CD19+ B cells or more restrictedly, in IgG1+ germinal center (GC) B cells. Within three months, TG mice developed splenomegaly with erased GCs and defective Ig class switch recombination (CSR) and affinity maturation. Within 20 months, most TG animals developed histiocytic/DC sarcoma of B cell origin.

Although these vFLIP TG mice did not develop tumors faithfully mimicking either human MCD or PEL, a number of striking findings were made. First, an enrichment of IgMκ-expressing plasmablasts occurred in all mice. The findings in these transgenic mice support the conclusion that KSHV latent transcripts, including vFLIP, are responsible for the preferential expansion of IgMκ+ cells. This is supported by the findings of Hassman et al., who showed that KSHV selectively established latent (LANA+) infection almost exclusively in Igκ B cells, despite the fact that both Igk and Igκ B lymphocytes expressed the lytic viral protein PAN after exposure of CD19+ lymphocytes to KSHV (3), and by the discovery that λ-chain–positive B cell development is dependent on NF-κB (21). Second, the Ballon et al. study reveals the in vivo immunological consequences of vFLIP when expressed in B cells. The abrogation of GC formation and inhibition of CSR and affinity maturation by vFLIP could directly contribute to KSHV pathogenesis by curtailing host immunity. Third, this work indicates the in vivo capacity of vFLIP to reprogram/transdifferentiate B cells into histiocytes. This finding supports the clonal B cell origin of histiocytic sarcoma, which can develop in individuals with B cell lymphoproliferations (22), including KSHV-related MCD, and argues that NF-κB signaling contributes directly to the in vivo plasticity of B cells.

**Future directions**

These three studies are sound foundations for further investigations of KSHV infection in vivo. Humanized mouse models could be used to test the in vivo relevance of the studies by Myoung et al. and Hassman et al. and to decipher the molecular mechanisms favoring IgMκ B lymphocyte expansion and B cell transdifferentiation (2, 3, 23). Furthermore, the vFLIP transgenic mice described by Ballon et al. could be crossed with mice expressing other KSHV latency–associated transcripts and complement the development of inducible TG mice expressing more than one latent transcript (3). We
could also speculate that the activation of vFLIP (with or without other latent viral transcripts) at a more mature stage of B cell differentiation, such as in post-GC B cells, will permit completion of the GC reaction and better recapitulate KSHV-lymphoproliferation development. These three studies are opening up new avenues to explore the immunobiology of KSHV as it relates to its principal reservoir, B lymphocytes.

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Adherent-invasive *E. coli* in Crohn disease: bacterial “agent provocateur”

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The role of adherent-invasive *E. coli* (AIEC) in Crohn disease (CD) has been in debate for decades. AIEC bacteria are found in the small intestine of patients with chronic CD, but it has remained unclear whether this infection is causal or secondary to underlying immune deficiencies in CD patients. In this issue of the *JCI*, Chassaing and colleagues demonstrate that AIEC bacteria express an adherence factor called long polar fimbriae (LPF) that aids in the binding of these bacteria to M cells overlying Peyer’s patches and subsequent entry into lymphoid tissue. These findings provide a mechanism of AIEC penetration but do not prove that AIEC is causing a primary infection in the Peyer’s patches that is necessary for the initiation or persistence of CD inflammation.

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Invasive *E. coli* as a cause of Crohn disease: a trail of research
The concept that Crohn disease (CD) is due to an infectious organism has been under investigation since this inflammation was first distinguished from mycobacterial infection some 80 years ago. While in recent decades enthusiasm for this concept has waned in the face of evidence that the disease is due to a dysregulated (and excessive) immune response against one or more commensal organisms in the intestinal microflora, creditable research is still being conducted to establish its validity. By far the most impressive example of this work is that of Darfeuille-Michaud and colleagues, who have accumulated a large body of data showing that an *E. coli* organism may be involved in CD pathogenesis. The basic findings of these investigators are as fol-