Human herpesvirus-4 or Epstein-Barr virus (EBV) is transmitted between humans by contact with saliva. The main target cells are B cells that express CD21 which serves as a primary entry receptor as well as HLA class II molecules that serve as a co-receptor. The virus can also infect epithelial cells and may initially replicate in the oro-pharynx before infection of B cells. Naive B cells become infected in the mucosal lymphoid tissues, particularly the tonsils and establish pools of latently infected memory B cells that recirculate to other lymphoid tissues. Periodic reactivation of latently infected memory B cells are thought to facilitate infection of epithelial cells in the oro-pharynx that permits shedding of virus into saliva necessary for transmission to new hosts. Latently infected memory B cells are long-lived and due to low levels of expression of viral proteins they escape detection by CD8+ cytotoxic T cells. Therefore persistence of EBV in the body is lifelong.
The normal activation pathway of naive B cells takes place in the lymphoid follicles of secondary lymphoid organs where B cells contact antigens displayed on follicular dendritic cells. Recognition of antigen by the B cell receptor results in endocytosis of the antigen-receptor complex followed by antigen processing and display of peptides on HLA class II receptors. B cells then migrate to the edge of the follicle where they contact effector CD4+ T cells that provide activation signals. The activated B cell migrates back into the follicle and proliferates into centroblasts. Centroblasts interact with CD4+ follicular helper T cells that promote affinity maturation and isotype-switching. Some of the B cells then differentiate into long-lived memory cells while others become antibody-secreting plasma cells. EBV uses this activation pathway to generate latently infected memory B cells that serve as a long-term reservoir for the virus.
EBV readily infects naive B cells due to the expression of CD21 and HLA class II receptors on the cell surface. The virus envelope protein gp350 binds to the primary receptor CD21 and gp42 binds HLA class II receptors that facilitates fusion of the virus membrane with the cell membrane. Infection of naive B cells results in expression of a set of viral latency genes that transforms the B cell and prevents apoptosis. Expression of viral membrane protein LMP1 mimics CD40-CD154 (CD40L) signal transduction and bypasses the need for CD4+ T cell help. Expression of viral membrane protein LMP2A mimics B cell receptor signaling. Together this permits the B cell to differentiate into memory and effector B cells. After initial B cell expansion (driven by EBV latency genes), most latency genes are switched off to evade detection by CD8+ T cells. LMP2A additionally prevents the B cell receptor from becoming activated since this can trigger the lytic pathway.
EBV infection of B cells generates a subset of cells that are actively producing infectious virus particles. This is the lytic cycle and these cells are readily killed by CD8+ cytotoxic T cells due to recognition of HLA class I receptors with bound viral peptides. In infectious mononucleosis there is an expansion of EBV-specific CD8+ T cells that eliminate virus-infected B cells. Latent infection of memory B cells is associated with reduced expression of viral proteins and hence these cells are not easily detected by CD8+ T cells and serve as a reservoir of EBV that persists for life. The lytic cycle can be induced in latently infected B cells by triggering of the B cell receptor. This occurs periodically and facilitates shedding of virus in saliva which can then be transmitted to new hosts.
In order for B cells to differentiate into plasma cells and memory cells it is essential for CD40 signal transduction to occur. CD4+ follicular helper T cells provide CD154 (CD40L) engagement following interaction with HLÃ class II molecules on the B cell surface. CD40 activation leads to signal transduction events that activate gene transcription. Signal transduction is mediated by adaptor molecules that bind to the intracellular tail of the CD40 receptor. These include JAK3 that initiates activation of STAT transcription factors and TRAF6 that facilitates NFkB activation and also the AP-1 activation pathway. These transcription factors migrate to the nucleus and initiate gene transcription. EBV circumvents the need for CD40 signaling by providing a homologue molecule LMP1 that mimics CD40.
EBV encodes a membrane protein LMP1 that is a functional homologue of CD40. The cytoplasmic tail binds adaptor molecules similarly to the CD40 cytoplasmic tail and allows signal transduction to take place in the same way as CD40-CD154 (CD40L) engagement. The LMP1 molecule is constitutively active and does not require ligand binding. This molecule mimics CD40-CD154 (CD40L) engagement normally provided by CD4+ follicular helper T cells that activates B cells and promotes affinity maturation, isotype switching and differentiation into plasma cells and importantly memory cells that the virus uses as a long-term reservoir.
Naive B cells contact antigen displayed on follicular dendritic cells in the lymphoid follicles of secondary lymphoid organs. The B cell receptor mediates signal transduction events via adaptor molecules that attach to the alpha and beta chains in association with the B cell receptor. These adaptors include LYN and SYK. SYK activates phospholipase C gamma-2 that generates IP3 and DAG. IP3 induces calcium influx that via calmodulin-calcineurin interaction activates NFAT transcription factors. DAG activates the NFκB pathway via protein kinase C-theta interaction. Membrane PI3K can also interact with PIP2 to generate PIP3 which activates NFκB via the AKT pathway. These transcription factors then initiate gene transcription in the cell nucleus. EBV encodes LMP2A, a homologue molecule of the B cell receptor, which can provide similar activation signals. Additionally LMP2A prevents normal B cell receptor signal transduction since this can trigger the lytic cycle.
EBV encodes membrane protein LMP2A, a functional homologue of the B cell receptor. The cytoplasmic tail has binding sites for the adaptor molecules LYN and SYK that bind the alpha and beta chains associated with the B cell receptor. Activation of SYK provides downstream signaling that B cell receptor engagement by antigen binding provides. LMP2A is constitutively active and does not require ligand or antigen binding. The LMP2A signal coupled with LMP1 signaling bypasses the need for antigen stimulation and help from CD4+ follicular helper T cells and promotes affinity maturation, isotype switching and differentiation into memory B cells harboring latent EBV and plasma cells. LMP2A also prevents the B cell receptor from activation by antigen since this triggers the lytic cycle and enhance immune detection. LMP2A achieves this by sequestering available LYN and SYK adaptor molecules and additionally accelerates their destruction via the ubiquitin degradation pathway since the cytoplasmic tail also recruits ubiquitin ligases.
Infectious mononucleosis caused by EBV is associated with an increase in peripheral blood lymphocytes (lymphocytosis). This is due to proliferation of EBV-transformed B cells as well as clonal expansion of EBV-specific CD8+ cytotoxic T cells. EBV transformation of B cells also leads to polyclonal B cell activation and antibody production which can generate immune complexes associated with immune-mediated skin rashes.
During acute infectious mononucleosis viral gene expression in both latently and lytically infected B cells induces a vigorous cell mediated immune response involving CD8+ cytotoxic T cells which can lead to increased levels of pro-inflammatory cytokines. High levels of INF-γ and TNF-α can induce excessive activation of macrophages and monocytes. In genetically predisposed individuals the enhanced phagocytic ability of activated macrophages is often associated with destruction of haematopoietic cells or their precursors in bone marrow, spleen or lymph nodes (haemophagocytosis) causing cytopaenia. Activated macrophages also secrete pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α, that can cause fever, rash (increased vascular permeability) and multiple organ infiltration by immune cells. Failure to control EBV replication can lead to death due to multiple organ failure.
A cell-mediated immune response to both lytic and latent antigens is required to control EBV infection. More specifically a CD8+ cytotoxic T cell response. CD8+ cytotoxic T cells have reduced ability to detect latent infected B cells due to the lower expression levels of viral proteins which is a survival strategy of the virus whereas lytic infections of B cells are rapidly destroyed. CD8+ cytotoxic T cells detect viral peptide antigens displayed on HLA class I receptors on the surface of infected B cells. Activation signals are transduced via the TCR. Additional activation signals are provided by SLAM-related membrane receptors NTB-A and 2B4 and their respective ligands NTB-A and CD48 when the membranes of the T and B cells contact. Signal transduction is mediated by the SAP adaptor molecule that recruits FYNT and provides activation signals. In XLP, mutations in the SAP adaptor molecule leads to a failure of EBV-specific CD8+ cytotoxic T cell killing of infected B cells. In the absence of SAP, inhibitory signals are transduced by the SLAM-related receptors.
CD8+ T cells detect infected target cells by engagement of the TCR with HLA class I receptors on the cell surface. In EBV infection, because the target cell is a B cell, there are additional interactions of cell surface molecules such as the SLAM-related receptors. B and T cells express many of these receptors, but NTB-A and 2B4 are important in mediating additional activation signals to the CD8+ T cell when engaging with ligands NTB-A and CD48. CD48 is upregulated on the surface of EBV-infected B cells. In XLP, the SAP adaptor protein that associates with the SLAM-related receptor is non-functional and fails to provide activation signals. It is thought that in the absence of functional SAP, the EAT-2 adaptor molecule associates with the SLAM-related receptors and this adaptor provides an inhibitory signal to the CD8+ T cell and leads to dysfunctional killing of infected B cells. Interestingly, this phenomenon is particular to EBV, since control of other viral infections is not compromised.