Epstein–Barr virus (EBV) was first identified in malignant Burkitt lymphoma cells in 1964. Since then, EBV has been associated with a number of other malignancies of either lymphocytic origin, including both B cell and NK/T cell cancers, or epithelial origin, predominantly nasopharyngeal and gastric cancers. While a complete understanding of the relationship between EBV-mediated cellular transformation and the oncogenic events that lead to uncontrolled malignant cell growth remains to be determined for a number of these cancers, it is clear in all of these settings that a breakdown in the immune surveillance of virally infected cells contributes to the survival of EBV-bearing malignant cells.

The lifecycle of EBV infection

As with most members of the Herpesvirus family, evolutionary adaptation over millions of years has allowed EBV to establish a unique biological niche in humans that allows it to maintain persistent infection for life with typically limited complications for the host and an efficient capacity to infect a new host. As a consequence of this efficient lifecycle, EBV is ubiquitous in the community, infecting 90–95% of the world population. EBV infection typically occurs via the mucosal surfaces of the oropharynx. Primary lytic infection is followed by the establishment of latent infection of B lymphocytes. Following transition of the linear EBV genome to the nucleus of the B cell, the EBV chromosome is circularised. Latent gene expression is then initiated from the W promoter, inducing the expression of the EBV nuclear antigens (EBNA) 2 and 5, which then induce the expression of other EBNA proteins, including EBNA1, 3, 4 and 6, and the latent membrane proteins (LMP) 1, 2a and 2b. While all of the latent cycle genes play important roles in latent transformation, EBNA1 is indispensible for viral latency and functions by binding to the origin of replication, oriP, on the viral episome. EBNA1 tethers the viral episome to the host chromosome, promoting maintenance of the latent EBV episome in daughter cells. Intermittent lytic replication in the upper respiratory tract promotes viral shedding and spread to a new host. This unique aspect of EBV biology, and that of the related gamma-herpesviruses, distinguishes it from other herpesviruses by promoting B cell proliferation without the need for active viral replication. However, it is also this capacity to efficiently transform B cells and induce lymphoproliferation, which leads to both the malignant and lymphoproliferative disorders, that are associated with EBV-infected cells.

Post-transplant lymphoproliferative disorders

Post-transplant lymphoproliferative disorders (PTLD) arise in immunocompromised patients who have undergone either solid organ (SOT) or haematopoietic stem cell transplantation (HSCT) and are almost universally associated with EBV infection. Under normal immunological conditions EBV-infected B cells are controlled by EBV-specific cytotoxic T lymphocytes, which efficiently clear these cells predominantly via the recognition of peptide epitopes encoded by EBNA3. However, the immunosuppressive environment associated with SOT and HSCT disrupts this immunological balance and can lead to uncontrolled proliferation of these EBV-infected cells that can typically be characterised with a latency III profile and the expression of the full array of latent genes (Table 1). PTLD in SOT patients is usually of recipient origin and is most prevalent in seronegative transplant recipients as a
consequence of primary infection following transplant of organs containing EBV-infected cells. However, PTLD can occur in sero-positive recipients as a consequence of the immunosuppressive regime used to prevent organ rejection. In Australia, PTLD is typically diagnosed in 1–10% of SOT recipients and is more highly prevalent in children due to an increased likelihood of seronegative status prior to transplant. PTLD in HSCT patients is usually of donor origin and is most prevalent in seropositive recipients who receive a transplant from a seronegative donor. This is typically facilitated by the reactivation of EBV of recipient origin and subsequent infection of B cells of donor origin and the concomitant immunosuppression of the induction of an EBV-specific cellular immune response. However, PTLD does occur following transplant from seropositive donors and increased risks are also associated with T cell depleted HSCT, commonly used to reduce the risk of graft-versus-host disease and due to HLA mismatch between donor and recipient.

A reduction in the immunosuppressive regime is usually the first option for treatment of PTLD in both SOT and HSCT patients in order to restore/promote EBV-specific cellular immunity. Rituximab, an antibody that recognises CD20 on the surface of most B cells, is also used to treat PTLD in both SOT and HSCT patients, often in combination with standard chemotherapy used to treat other B cell lymphomas. In addition, adoptive cellular therapy is emerging as a powerful tool for the treatment of EBV-associated PTLD, particularly in the context of HSCT. Pioneered by Rooney and colleagues at the Baylor College of Medicine in Texas, the transfer of EBV-specific T cells, which classically involves the use of in vitro-generated EBV-infected lymphoblastoid cell lines (LCL) to stimulate donor memory T cells, have been used effectively both therapeutically and prophylactically to treat or prevent PTLD in HSCT patients. However, in the context of PTLD in SOT patients or following a seronegative HSCT transplant, the success of EBV-specific cellular therapy is dependent on the generation of recipient-derived T cells (Table 1). While this has been used successfully to resolve PTLD in SOT patients, an emerging approach for the treatment of PTLD in these instances is the use of a bank of third-party T cells. These T cells are generated from another EBV-seropositive healthy donor who shares HLA alleles with the patient. Initial reservations surrounding the potential risk of inducing graft-versus-host disease or organ rejection have proven to be unfounded following the administration of third-party T cells and they have proven effective in the treatment of PTLD in both SOT and HSCT patients.

EBV-associated Hodgkin and non-Hodgkin lymphomas

In Australia, Europe and North America, 30–50% of Hodgkin lymphoma (HL) cases are associated with EBV. In contrast, EBV positivity in HL cases reaches as high as 100% in certain regions of Asia, Africa and Latin America. A unique characteristic of HL is the large inflammatory infiltrate that is associated with lymphoid organs in HL patients. The malignant Reed–Sternberg cells in HL comprise less than 1% of the cellular mass in inflamed lymphoid organs and the nature of the infiltrate is used histologically to characterise the HL

<table>
<thead>
<tr>
<th>EBV malignancy</th>
<th>Latency profile</th>
<th>Association with EBV</th>
<th>Viral gene expression</th>
<th>EBV-related treatment in clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-transplant lymphomas (early and late onset)</td>
<td>Latency III</td>
<td>Early: predominantly EBV+</td>
<td>Early: EBNA 1-6, LMP1-2</td>
<td>Therapeutic/prophylactic administration of autologous, donor-derived or third party EBV-specific T cells</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>Latency I</td>
<td>Almost 100% in endemic regions</td>
<td>EBNA1</td>
<td>None tested</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>Latency II</td>
<td>30–50%</td>
<td>EBNA1, LMP1-2</td>
<td>Therapeutic administration of EBNA1/LMP1-2 T cells. Therapeutic administration of EBNA1/LMP vaccine</td>
</tr>
<tr>
<td>NK/T cell lymphomas</td>
<td>Latency II</td>
<td>Almost 100% in endemic regions</td>
<td>EBNA1, LMP2 LMP1 (+/-)</td>
<td>None tested</td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>Latency II</td>
<td>Almost 100% in endemic regions</td>
<td>EBNA1, LMP1-2</td>
<td>Therapeutic administration of EBNA1/LMP1-2 T cells. Therapeutic administration of EBNA1/LMP vaccine</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>Latency I/II</td>
<td>10%</td>
<td>EBNA1, LMP2</td>
<td>None tested</td>
</tr>
</tbody>
</table>
EBV infection is most prevalent in classical HL. EBV-infected Reed–Sternberg cells express a latency II gene expression pattern, which is restricted to EBNA1 and LMP1&2 (Table 1), both of which play important roles in the transformation of infected B cells\textsuperscript{22,23}. In addition to their important role in the maintenance and transformation of EBV-infected B cells, EBNA1 and LMP1&2 are poorly immunogenic, relative to the other EBV-latent molecules. T cells specific for EBNA1&LMPI display a loss of function during acute HL and an increased susceptibility to immunomodulatory molecules generated by HL cells, such as galectin-\textsuperscript{24–26}. These immune evasion strategies are likely important for the maintenance of EBV-infected B cells in immune-competent hosts, but also provide mechanisms for immune evasion by malignant Reed–Sternberg cells\textsuperscript{27}.

The incidence of HL is bimodal in nature, with a peak in incidence in children under 10 years and in adults over 50 years. An increased risk of developing EBV-associated HL has also been linked to infectious mononucleosis during primary EBV infection\textsuperscript{28}. HL is highly amenable to current chemotherapeutic regimes, with 5-year survival rates approaching 90%. However, EBV infection is associated with more rapid progression in HL patients and reduced overall survival\textsuperscript{29}. The current chemotherapeutic regimes used to treat HL are also associated with an increased risk of the development of secondary cancers and strategies to reduce the dependence of the current level of chemotherapy are under development, particularly for children\textsuperscript{30}. EBV-associated HL, often when refractory to chemotherapy, has also been successfully treated with adoptive cellular therapy\textsuperscript{29,31–36}. While initial studies focussed on the LCL-based approach used to treat PTLD, more recent approaches have specifically targeted the induction of T cells specific for EBNA1 and LMP1&2\textsuperscript{37}. These approaches have proved to be successful in phase I clinical studies and are currently being evaluated in phase II studies\textsuperscript{31}.

EBV infection is also associated with a number of non-Hodgkin’s lymphomas (NHL), including Burkitt lymphoma (BL) and diffuse large B cell lymphoma (DLBCL)\textsuperscript{38}. Although rare in Australia, EBV-associated BL is endemic in regions of Africa and Papua New Guinea\textsuperscript{22}. It arises primarily in young children and its incidence is closely associated with malaria endemic regions, which has been attributed to a loss of functionality in EBV-specific T cells in children with co-exposure to malaria\textsuperscript{39,40}. Burkitt lymphoma cells typically display an EBV latency I profile, characterised by the expression of only EBNA1 (Table 1). In association with reduced expression of surface HLA molecules, this renders BL cells highly immunoevasive\textsuperscript{41–43}. Burkitt lymphoma is also characterised by the translocation of the gene encoding the oncogenic c-MYC protein\textsuperscript{44}.

**Nasopharyngeal carcinoma**

EBV infection is associated with the majority of undifferentiated nasopharyngeal carcinoma (NPC)\textsuperscript{45–47}. While rare in the Australian population, with an incidence of less than 1 in 100,000, EBV-associated NPC is endemic in regions of south-east Asia and North Africa. It reaches its highest prevalence in southern China with incidence rates of 15–50 cases per 100,000 within some communities\textsuperscript{48}. An increased incidence of NPC is also evident in Australia in immigrant populations from these regions; however, this incidence is reduced in first generation Australians, indicative of the role both genetics and environment play in the development of NPC.

Similar to HL, EBV gene expression in malignant NPC cells is restricted to EBNA1 and LMP1&2. Current treatment options for NPC typically involve surgical intervention, radiotherapy and/or chemotherapy\textsuperscript{49}. Standard treatment options have seen response rates in primary disease improve to greater than 90%. Despite this, a significant proportion of patients, particularly those who initially present with late stage disease, will relapse, which often leads to distance metastases that are refractory to conventional therapy\textsuperscript{49}. EBV-targeted treatment options are therefore in development as adjunct approaches to current radio/chemotherapeutic regimes. While some strategies focus on the induction of lytic viral reactivation as a potential mechanism of enhancing cellular immunity and inducing lysis of malignant cells, the majority of these approaches focus on using EBV-specific immunotherapy to treat NPC\textsuperscript{50} (Table 1). Augmentation of cellular immunity to EBNA1 and the LMP antigens have emerged as potential adjunct or alternative approach to chemo/radiotherapy. A number of phase I clinical studies treating NPC with a range of immunotherapeutic strategies have provided evidence that these approaches are safe and might have some clinical benefit, particularly in patients with locoregional disease\textsuperscript{50–52}. Current efforts are being placed on validating these observations in larger cohorts of patients.

**Other EBV-associated malignancies**

EBV-infection has also been associated with a number of other malignancies of both lymphocytic and epithelial cell origin, including a range of NK/T cell lymphomas, lymphoepithelial-like carcinomas and gastric carcinomas. While not considered a normal target for EBV infection, EBV is associated with a subset of lymphomas of NK and T cell origin\textsuperscript{51}. The most prevalent of these lymphomas are the nasal NK/T lymphomas that are rare in Australia, but more common in parts of south-east Asia. EBV-associated NK/T cell lymphomas have a very poor prognosis, with current 5-year survival rates of less than 50%\textsuperscript{51}. EBV infection in NK/T cell lymphomas is characterised with a latency I/II gene expression profile,
characterised predominantly by the expression of EBNA1 and LMP2\textsuperscript{52} (Table 1). Despite this, due to the lack of an in vitro model, the role EBV and these latent antigens play in the tumourgenesis of NK/T cell lymphomas is not clear.

The EBV-associated lymphoepithelial-like carcinomas share features with NPC but are found in a range of other anatomical sites, including the stomach\textsuperscript{53,54}. These cancers also show a distinct geographical distribution and are endemic in regions of Asia and indigenous communities in America, further evidence for the influence of environment and genetics on the incidence of EBV-associated carcinoma. EBV-infection is also associated with a small percentage of non-lymphoepithelial-like gastric carcinoma cases\textsuperscript{55}. While the incidence of gastric carcinoma has reduced significantly in most developed countries, the incidence remains high in developing countries and in some developed countries, including Japan and Korea\textsuperscript{56}. EBV infection in gastric carcinoma cells is associated with a geographical distribution and are endemic in regions of Asia and indigenous communities in America, further evidence for the influence of environment and genetics on the incidence of EBV-associated carcinoma. EBV-infection is also associated with a small percentage of non-lymphoepithelial-like gastric carcinoma cases\textsuperscript{55}. Although no aetiological relationship has been established between EBV infection and the transformation of gastric carcinoma cells, it will be important to explore therapeutic options that specifically target EBV to improve clinical outcome\textsuperscript{59}.

**Concluding remarks**

Over the past two decades considerable knowledge has been accumulated on EBV pathogenesis in different malignancies. This knowledge has recently been successfully exploited to develop new diagnostic and therapeutic tools that have significantly impacted on the clinical management of patients with EBV-associated diseases.

**References**

Biographies

Dr Corey Smith completed his PhD in 2004 at the University of Melbourne and then took up a research position in the Tumour Immunology Laboratory at the Queensland Institute of Medical Research where his work focuses on the development of immune-therapeutic approaches to treat cancers associated with viral infection. This work led to a phase I clinical study in Hong Kong, using a novel immunotherapeutic approach to treat Epstein–Barr virus associated nasopharyngeal carcinoma. His work has also focused on understanding the mechanisms that influence the efficient induction of T cell responses to persistent human viral infections and the role immune evasion strategies developed by virally associated proteins play in promoting the survival of infected malignant cells.

Professor Rajiv Khanna obtained his doctorate degree from India and undertook his post-doctoral training at the Queensland Institute of Medical Research (QIMR), Brisbane, Australia. He is currently appointed as the Coordinator of the Centre for Immunotherapy and Vaccine Development at QIMR and also holds Senior Principal Research Fellowship from the National Health and Medical Research Council of Australia. The major goals of his research group are to obtain a deeper understanding of the mechanisms by which an immune response to human herpesvirus-associated diseases might be generated, augmented and applied to control these diseases. Over the past few years, Professor Khanna has successfully translated his research towards the development of novel immune-based therapeutic strategies for the treatment of patients with herpesvirus-associated diseases and is currently lead investigator on five clinical trials. He has published more than 150 scientific papers in leading journals and holds numerous international patents on EBV and human cytomegalovirus (CMV) and has successfully co-developed a diagnostic kit (QuantiFERON-CMV) in collaboration with Cellestis Ltd (now Qiagen). QuantiFERON-CMV has been CE marked in Europe for diagnostic application and is expected to be launched in the US in late 2013. Currently, he is also collaborating with Intercell AG (now Valneva SE) to develop a new prophylactic vaccine against CMV.