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active Epstein–Barr virus infection', a recurrent febrile condi- tion that resembles persistent infectious mononucleosis; best known among Asian children, this again can progress to severe or fatal haemophagocytosis. Cancer Epstein–Barr virus is strongly linked to several different human can- cers. These include at least three types of B-cell malignancy, Burkitt lymphoma, lymphoproliferative disease/lymphoma of the im- munocompromised, and Hodgkin lymphoma. Non-B-cell tumours include extranodal lymphoma of T or natural killer cell origin, un- differentiated nasopharyngeal carcinoma, and a subset of gastric carcinomas. Together these constitute a global burden of 200 000 Epstein–Barr virus-positive cancers per year. B-cell malignancies 'Endemic' Burkitt lymphoma—all cells of this common malignancy of children in equatorial areas of Africa and New Guinea carry the Epstein–Barr virus genome. A cofactor, hyperendemic malaria, ex- plains the unusual geographical distribution of the high-incidence disease. Presentation is in the jaw or, less frequently, in the abdomen; peripheral lymph nodes and spleen are spared. The tumour grows re- markably quickly but is very sensitive to cyclophosphamide treatment. An essentially similar tumour, 'Sporadic' Burkitt lymphoma occurs worldwide but at much lower incidence and with only a minority of tumours being Epstein–Barr virus genome-positive. The sporadic disease presents more often in the abdomen than the jaw, and lymph nodes can be involved; the response to treatment is poorer and com- bination therapy is required, and survival after relapse is uncommon. A third form, 'AIDS-associated Burkitt lymphoma', occurs at un- expectedly high frequency in individuals with HIV infection, often as one of the first AIDS-defining symptoms and before overt im- mune impairment; some 30–40% of such tumours are Epstein–Barr virus-positive. Besides Burkitt lymphoma, Epstein–Barr virus is firmly linked to two other types of B-cell malignancy. One is lymphoproliferative disease/lymphoma of the immunocompromised, typically seen in transplant patients in the first year after transplantation when immune impairment is most severe, and also in late-stage AIDS patients; such lesions consist of Epstein–Barr virus-positive cells growing out op- portunistically in the absence of effective immune surveillance. The other B-cell tumour is Hodgkin lymphoma, where the virus genome is present in all the Reed–Sternberg and mononuclear tumour cells in

 about 30–40% of cases seen in the Western world and in a somewhat higher proportion of cases elsewhere. Non-B-cell malignancies and other conditions associated with Epstein–Barr virus Undifferentiated nasopharyngeal carcinoma—this epithelial tu- mour occurs in all racial groups but is most common in southern Chinese and Inuit people. All cases worldwide are Epstein–Barr virus genome-positive. Besides virus infection, both genetic and environ- mental (dietary and perhaps herbal remedy) cofactors are involved in tumourigenesis. Radiotherapy, now combined with chemotherapy, is the treatment of choice. A histologically similar carcinoma of the sal- ivary gland, most often observed in Inuit people, is also consistently Epstein–Barr virus genome-positive and is regarded as a variant of nasopharyngeal carcinoma. In addition, some 8–10% of gastric carcinomas around the world carry the Epstein–Barr virus genome. These Epstein–Barr virus- positive tumours show different cellular genetic changes from those seen in other forms of gastric carcinoma and have a slightly better re- sponse to treatment, suggesting that they represent a distinct subset of the disease. Epstein–Barr virus is also very strongly linked to a highly aggressive extranodal lymphoma of T- or NK-cell origin, sometimes presenting as a disfiguring nasal lesion, other times as a rapidly growing NK- cell leukaemia. The lymphoma is 100% Epstein–Barr virus genome- positive. Responses to chemotherapy are currently very poor. Besides being present in certain AIDS lymphomas, Epstein–Barr virus is also linked to two other HIV-associated conditions. One is a very rare tumour of smooth muscle cell origin, leiomyosarcoma

of AIDS, which is Epstein–Barr virus-positive in all cases. The other is a nonmalignant wart-like lesion of the oral cavity, oral hairy leukoplakia, which is caused by the virus actively replicating as a lytic infection of squamous epithelial cells. Both conditions are markers of the profound immune impairment seen in late-stage AIDS; accord- ingly, both show much reduced incidence following the introduction of highly active antiretroviral therapy. More controversially, Epstein–Barr virus has been linked with cer- tain autoimmune diseases. In particular, there is strong serologic and epidemiologic evidence to suggest that previous exposure to Epstein–Barr virus markedly increases the risk of developing multiple sclerosis. Although the Epstein–Barr virus/multiple sclerosis connec- tion is receiving much attention, the mechanism that might underpin such an association remains uncertain. Background The virus Epstein–Barr virus (EBV) was discovered in 1964 during a sus- tained search for a viral cause of endemic Burkitt lymphoma (see ‘Endemic (‘African’) Burkitt lymphoma’, next). EBV is one of eight human herpesviruses and the only one belonging to the γ-1 sub- family. It has a typical herpesvirus structure, with an outer envelope and a protein capsid shielding the inner core with its large double- stranded linear DNA genome. EBV clearly has a long coevolutionary history with our species as related γ-1 viruses have been found in both Old World apes and monkeys and in New World monkeys. This indicates that a common ancestor of all contemporary γ-1 viruses must have been present in early primate species before the evolu- tionary split between Old World and New World primates occurred (about 35 million years ago based on palaeontology, or 45 million years ago based on DNA sequence analysis). Viral infections, lytic and latent On transmission to a naïve host, herpesviruses typically replicate in one or more cell types as a lytic infection, producing thousands of copies of new infectious virions but killing the host cell in the process, then establish a latent (antigenically silent) infection in one particular cell type. This reservoir of latency allows the virus to escape detection and survive for years in the immune-competent host. EBV accords to this pattern, replicating in oropharyngeal epi- thelium and probably also locally infiltrating B cells, before going latent in the recirculating memory B cell pool. In addition, however, the virus has evolved a unique set of protein-coding latent genes which, when expressed collectively, are able to drive B-cell growth. In the newly infected host, EBV uses this growth-transforming ability to expand the number of infected cells invading the B-cell system, before switching off latent gene expression in those cells and going silent in memory B cells. The same growth-transforming ability can also be seen in vitro when the virus infects B cells and drives them into continuously growing, latent antigen-expressing lymphoblastoid cell lines. Virus-coded proteins and the immune response Different sets of virus-coded proteins are expressed in lytic and latent infection. There are over 60 EBV-coded proteins expressed during lytic cycle, and these are categorized as immediate early, early, or late antigens, according to the order in which they appear. By contrast, growth-transforming infection involves the coordinated expression of eight unique latent proteins. Both lytic and latent proteins elicit a range of antibody and cell-mediated immune responses that help to bring the initial infection under control; those responses then mature and persist for life. Assays for serum antibodies to repre- sentative lytic and latent proteins can therefore be used to identify individuals who have never been infected by EBV, those undergoing primary infection, and those with an established carrier state. Epidemiology The virus is widespread in all human populations. Primary infection usually occurs in early childhood, when it is almost always clinic- ally silent. This leads to a lifelong carrier state, in which the virus persists as an antigenically silent, latent infection in a small number of memory B cells. Reactivation of the virus infection is normally contained by the host’s humoral and cellular immune responses. However, low level reactivations into lytic cycle from recirculating latently infected B cells are thought to seed subclinical foci of lytic- ally infected epithelial cells in the mouth and pharynx, and

perhaps also in the salivary glands. This leads to recurrent low-level shedding of infectious virus in the buccal fluid of virus carriers, allowing oral transmission of the infection within the population. In developing countries, 99% of children are infected by the second to the fourth year of life. By contrast, in industrialized countries with higher standards of hygiene, as many as 50% of children, particularly those

 from high socioeconomic groups, enter adolescence uninfected (Fig. 8.5.3.1). Infectious mononucleosis Careful prospective studies on student populations report that up to 80% of those who first acquire the virus in the second or third decade develop some clinical symptoms of infectious mononucle- osis. However the severity of symptoms varies greatly between in- dividuals and it is likely that only a minority of the aforementioned will be sufficiently ill to consult a doctor. Given its strong associ- ation with delayed infection, mononucleosis is therefore mainly a disease of upper socioeconomic groups in Western societies, and is exceptionally rare in developing countries (Fig. 8.5.3.1). Although most cases occur in adolescents and young adults, children and older people might sometimes develop the disease. Primary in- fection in adolescence or later is likely to be acquired by kissing a virus-shedding healthy carrier. This explains why case-to-case in- fection and epidemics are not seen, and why the incubation period, estimated as 30 to 50 days, is difficult to calculate. Symptomatic pri- mary EBV infection can also be acquired through latently infected B lymphocytes present in blood transfusions or organ grafts, where the donor is an EBV carrier and the recipient is EBV-naïve. Symptoms Classical infectious mononucleosis might follow days of vague indisposition, or can start abruptly. It presents with sore throat, fever with sweating, anorexia, headache, and fatigue, with mal- aise quite out of proportion to the other complaints. Dysphagia might be noticed, and also brief orbital oedema. Erythematous and maculopapular rashes occur in a small number of untreated patients, but much more frequently in those that have been taking ampicillin for sore throat before infectious mononucleosis has been diagnosed (Figs. 8.5.3.2 and 8.5.3.3). Tonsillar and pharyn- geal oedema can rarely cause pharyngeal obstruction (Fig. 8.5.3.4) Signs The fever can rise to 40°C, but swings are not seen. There is red- ness and oedema of the pharynx, fauces, soft palate, and uvula (Fig. 8.5.3.4a), and about half the patients develop greyish ex- udates on the tonsils (Fig. 8.5.3.4b). Generalized lymphadenop- athy is almost always present, and is most marked in the cervical region; the glands are symmetrical, discrete, and slightly tender. Splenomegaly is seen in about 60% of cases and an enlarged liver in 10%. There is usually a moderate bradycardia. Besides the rash, characteristic palatal enanthematous crops of reddish petechiae (Fig. 8.5.3.4c) are found in about one-third of patients, and jaun- dice occurs in about 8%. Clinical course Mild cases might resolve in days, but 1 to 4 weeks is more usual, followed by a period of lethargy. The duration of this convalescence is influenced by psychological factors, particularly the speed with which patients are encouraged to resume full activity. About 1 case in 2000 might continue in a truly chronic or recurrent form for several months or years (see ‘Chronic active EBV infection’, next). Most other cases of so-called chronic infectious mononucleosis are manifestations of chronic fatigue syndrome (Chapter 26.5.4), but whether this is a true entity rather than a form of depression or a be- lief disorder is highly controversial. Credible connections with EBV have not been established. Africa SE Asia developed countries less well off developed countries affluent classes 100 75 50 25 0 Percentage of individuals infected with EBV Age (years) 70+ 60 50 40 30 25 20 15 5 10 Fig. 8.5.3.1 Comparison of the ages at which people in different populations become infected with EBV. In developing countries, almost all children have acquired the virus by 2 to 4 years of age, depending on geographical region. In developed countries with high standards of living and hygiene, the time of

infection is delayed for many, more markedly among the affluent than the less well off. Among the very rich, as many as 50% may reach adolescence or young adulthood without having encountered the virus, and will undergo delayed primary infection, with a high risk that this will be accompanied by the symptoms of infectious mononucleosis. Reprinted with permission from Epstein MA (2002). Infectious mononucleosis. In: Encyclopedia of life sciences, 10, 211–16. Copyright © 2001 John Wiley & Sons, Ltd. Fig. 8.5.3.2 Typical maculopapular erythematous rash in a patient with infectious mononucleosis who was treated with ampicillin. Copyright D. A. Warrell.

8.5.3 Epstein–Barr virus 757 Complications Minor nonspecific complications can occur. Rare, more ser- ious complications include secondary bacterial throat infections, traumatic rupture of the enlarged spleen, asphyxia from pharyn- geal oedema, massive hepatic necrosis, Guillain–Barré syndrome, and autoimmune manifestations such as thrombocytopenia and haemolytic anaemia. Differential diagnosis Classical infectious mononucleosis is diagnosed by the clinical fea- tures, combined with serological and haematological laboratory investigations (see next). An infectious mononucleosis-like disease can occur in primary cytomegalovirus infection and in toxoplas- mosis, but in both conditions the sore throat is much less severe, and with cytomegalovirus the lymphadenopathy might be minimal or absent; an infectious mononucleosis-like syndrome is also some- times seen with primary HIV infection. Laboratory diagnosis Several diagnostic methods have been developed and evaluated. These include (i) the Monospot test for heterophile antibodies, (ii) multiplexed bead-based assay (BBA), enzyme immunoassay (EIA), or immunofluorescence assay (IFA) for IgM/IgG antibodies to EBV viral capsid antigen (VCA) and EBV nuclear antigen 1 (EBNA1) and (iii) measurement of EBV viral genome load (EBV- VL) either in whole blood or in peripheral blood mononuclear cells using quantitative real-time polymerase chain reaction (QRT-PCR) assay. The Monospot screening test detects the pres- ence of heterophile antibodies in the patient’s serum. Although these heterophile antibodies are not directed against virally en- coded proteins, they are present in up to 85% of acute infectious mononucleosis sera; cases of Monospot-negative infectious mono- nucleosis tend to be outside the usual 15- to 25-year age range, and false-positive tests may occur in pregnancy and autoimmune dis- ease. The diagnosis of infectious mononucleosis can be confirmed by the presence of serum IgM antibodies to VCA, accompanied by rising IgG anti-VCA antibodies, in the absence of detectable IgG antibodies to EBNA1; patients remain IgM anti-VCA-positive for about 2 months while the IgG anti-EBNA1 response typically appears 3 months or more after the disease course. Eventually, the patient’s serological picture assumes that of the lifelong virus carrier state, that is IgM anti-VCA-negative, IgG anti-VCA-posi- tive, IgG anti-EBNA1-positive. The QRT-PCR assay is proving useful in the early diagnosis of infectious mononucleosis where EBV IgM immunoassays (multiplexed BBA, EIA, and IFA) prove inconclusive. Assays designed with EBV genomic probes within the BALF5 gene or within the BAMH1-W repeat region are highly sensitive. Note that the level of EBV DNA detected in infectious 100 75 50 25 0 Percentage of patients with clinical features Duration of IM (days) 28 21 14 7 Enlarged lymph glands Enlarged spleen Temperature ↑ Sore throat Spots on palate Jaundice Rash Swollen eyes Fig. 8.5.3.3 Percentage of patients with infectious mononucleosis showing various clinical features during the course of the disease, and the timing and average duration of each. Reprinted with permission from Epstein MA (2002). Infectious mononucleosis. In: Encyclopedia of life sciences, 10, 211–16. Copyright © 2001 John Wiley & Sons, Ltd. (a) (b) (c) Fig. 8.5.3.4 Infectious mononucleosis: (a) Oedema of fauces, soft palate, uvula, and tonsils; (b) tonsillar exudates; and (c) palatal petechiae. Courtesy of the late Dr B. E. Juel-Jensen.

758 section 8 Infectious diseases mononucleosis blood reflects the load of circulating latently infected B cells, not the load of free virus; levels of cell-free EBV DNA within plasma are very low and often undetectable even in acute disease. Other diagnostic features of the disease include the presence of lymphocytosis up to 15 × 109/litre, composed mainly of activated cytotoxic T cells (the 'atypical mononuclear cells' that gave mononucleosis its name), a decreased CD4/CD8 ratio (0.3), and raised C-reactive protein concentrations. Treatment Bed rest and aspirin or paracetamol for headache and pharyngeal discomfort are the only treatments required for most patients with infectious mononucleosis. The role of corticosteroids, other than in patients with unusually severe symptoms, is controversial. When the fever resolves the patient should be encouraged to get up and resume some activities as fast as is practicable, but violent exercise should be avoided for 3 weeks after an enlarged spleen ceases to be palpable. Only complications need active therapy; splenic rup- ture requires surgery, bacterial infections call for appropriate anti- biotics, airway obstruction must be relieved by tracheostomy, and corticosteroids should be given for life-threatening pharyngeal oedema, and for neurological and haematological complications. Immunocompetent patients with severe infectious mononucleosis have been treated with a variety of agents targeting lytic virus rep- lication (including aciclovir, valciclovir, famciclovir, ganciclovir with or without foscarnet, and vidarabine) together with cor- ticosteroids or intravenous immunoglobulin. The utility of such treatments remains unproven. Clinical evidence does not support the use of aciclovir alone but more patients receiving a combin- ation of antivirals and immunosuppressives survived compared to those given antiviral therapy alone. Suggested experimental treatments are 5-substituted uracil, azacytosine derivatives aimed at destroying cells entering lytic cycle and expressing EBV-coded thymidine kinase, and peptides inhibiting EBV-mediated mem- brane fusion. Pathogenesis Acquisition of orally transmitted virus leads to a phase of high level virus replication in the oropharynx, through lytic infection of epithelial cells and probably also intraepithelial B lymphocytes, followed by colonization of the general lymphoid system through a latent growth-transforming infection of B lymphocytes. In infec- tious mononucleosis patients, these combined lytic and latent in- fections stimulate an exaggerated cell-mediated immune response, characterized by mild NK-cell activation but large expansions of activated cytotoxic CD8+ T cells; these CD8+ T cells are found not just in the circulation but also in oropharyngeal lymphoid tissues such as the tonsils, as well as more generally in lymph nodes, spleen, and liver. This exaggerated response, and the cytokine storm that accompanies it, are thought to be responsible for the sore throat, fever, malaise, lymphadenopathy, and hepatosplenomegaly. In sup- port of this idea, in the few individuals who have been identified undergoing asymptomatic primary infection, the blood picture showed no lymphocytosis. Infectious mononucleosis is therefore an immunopathological disease. It is not known why the disease occurs frequently following primary EBV infection in adolescents and young adults, yet rarely in children; however, this must relate to circumstances that influence the size of the cell-mediated response. One possible factor is virus dose per se, with higher doses likely to be acquired by kissing in adolesecence/young adulthood. However, the issue of acquired dose remains contentious since recent work has de- tected EBV genome loads in the blood of asymptomatic primary infections that are the equal of those seen in infectious mono- nucleosis patients. Another possible factor is the age-dependent maturation of the human immune system, with NK-cell control over the very early phase of viral infection becoming less effi- cient with age, thereby placing more burden on the CD8+ T-cell response. X-linked lymphoproliferative disease (fatal infectious mononucleosis) (OMIM 308 240) An extremely rare genetically determined susceptibility to EBV occurs in young males of certain kindreds, who develop X-linked lymphoproliferative (XLP) disease following primary

infection. This presents initially with acute mononucleosis-like symptoms, but progresses inexorably to haemophagocytosis, which cul- minates in the necrotic destruction of vital organs, leading to multisystem failure. The mutated X-chromosomal gene (SH2D1A) responsible for this defect encodes a protein (SAP) that is involved in the normal regulation of T cell and natural killer (NK) cell re- sponses, in particular the ability of these cells to recognize and interact with B lymphocytes. This has several immunological con- sequences but is especially damaging with respect to combating EBV, a virus that is harboured in the B-cell system. In patients with XLP, EBV induces the same CD8+ T-cell and NK-cell responses as in classical infectious mononucleosis, but the responses are nei- ther able to control the B-cell infection, nor to receive the signals that would normally control their own expansion. As a result, XLP patients develop a highly exaggerated form of infectious mono- nucleosis, with even more dramatic CD8+ T-cell and NK-cell ex- pansions, very high loads of inflammatory cytokines and an often fatal haemophagocytosis. Chronic active EBV infection There are very rare cases of infectious mononucleosis that fail to re- solve, and may continue for years, often developing serious compli- cations leading to death. These cases of chronic active EBV infection can occur in both sexes, show no familial linkage, and are more common in people of Asian than European descent. Symptoms and signs Persistent fever, lymphadenopathy, and hepatosplenomegaly are frequently accompanied or followed by anaemia, thrombocyto- penia, and mononuclear cell haemophagocytosis. The disease can, therefore, lead to a clinical endpoint not unlike that seen in fatal XLP, but by a different pathogenetic route (see next). Pathogenesis and treatment Chronic active EBV infection is unique in that, in most cases, the disease is a consequence of unscheduled entry of the virus into T and/or NK cells; the circumstances which allow such atypical

 infection are not understood. The infected T or NK cells appear to escape normal immune controls and so proliferate, infiltrating vital organs and releasing the cytokines that are thought to initiate haemophagocytosis. Such infections also carry an oncogenic risk and, even within 1–2 years of first presenting, some of these patients develop a monoclonal EBV-positive T- or NK-cell lymphoma. There is no satisfactory treatment for this disease, but haemato- poietic stem cell transplantation is being evaluated with encouraging early results. Endemic (‘African’) Burkitt lymphoma The classical form of this B-cell tumour, first described by Burkitt in 1958, is found in those parts of Africa and Papua New Guinea where the temperature does not fall below 16°C, and the annual rainfall does not fall below 55 cm. Endemic Burkitt lymphoma is a disease of childhood, is extremely rare over the age of 14 years, and in endemic areas is more common than all other childhood tumours added together. The association between latent EBV infection and the cells of endemic Burkitt lymphoma is so close (virtually 100%) that it is generally accepted that the virus is essential, although it requires combination with cofactors in a complicated chain of events to lead to the malignancy. Hyperendemic malaria has been identified as an important cofactor, and its spread by anopheline mosquitoes re- quiring warmth and moisture explains the climate dependence. Symptoms and signs The endemic tumour is usually multifocal, and the symptoms de- pend entirely on the anatomical location. Jaw tumours are present in 70% of patients, are the usual presenting feature, may be multiple in all four quadrants, and are almost always accompanied by tumour foci elsewhere. The rapidly growing mass causes loosening of teeth, and exophthalmos from orbital spread. Abdominal tumours involve retroperitoneal nodes, liver, ovaries, intestines, and kidneys. Burkitt lymphoma sometimes presents in the thyroid, the adolescent fe- male breast, the testicles, and salivary glands; extradural tumours in the spine cause rapid paraplegia, and skeletal tumours also occur.

Characteristically Burkitt lymphoma does not involve the spleen or peripheral lymph nodes. The tumours are firm, very rapidly growing, painless, and cause minimal constitutional disturbance. Their site determines the clinical signs. Differential diagnosis In endemic areas, Burkitt lymphoma can be diagnosed from the clinical picture. Unlike the Burkitt tumour, retinoblastoma is intraocular; rhabdomyosarcoma is extraorbital, and does not in- volve teeth; nephroblastoma is not multifocal; and neuroblastoma and ovarian tumours can be distinguished histologically. Paraplegia of tuberculous origin causes vertebral collapse, and acute transverse myelitis is preceded by pain and fever. The anatomical distribution of other lymphomas is quite different. Laboratory diagnosis Histological examination of a biopsy sample is clearly diagnostic. Antibodies to EBV antigens show a distinct pattern, with IgG anti-VCA titres are around 10-fold higher than in matched controls, with antibodies to EBV-restricted early antigens and membrane antigens also detectable. Anti-VCA titres appear to reflect tumour load, rising with disease progression and falling with posttreatment remission; this implies that, although the tumour is latently infected, a small fraction of tumour cells in vivo spontaneously enter lytic cycle and release lytic proteins. Clinical course and treatment Tumour growth is relentless, and death ensues within a few months in the absence of treatment. Surgery and radiotherapy are ineffective, but moderate courses of chemotherapy give excellent results. This reflects the marked sensi- tivity of this rapidly growing tumour to cytotoxic drugs that induce programmed cell death (apoptosis). Cyclophosphamide, the drug of choice, remains effective after relapses. Pathogenesis Burkitt lymphoma is a monoclonal malignancy of germinal centre B-cell origin. EBV expresses a very limited range of latent genes in the tumour cells, with EBNA1 the only consistently detect- able viral antigen. When combined with a key cellular genetic change—a chromosomal translocation leading to overexpression of the MYC oncogene—this restricted form of virus latency ap- pears to complete the malignant conversion of the target cell, giving rise to the tumour. Cofactors such as hyperendemic mal- aria may contribute, both by chronically stimulating turnover of the target germinal centre cell population in the B-cell system, thereby increasing the chances of rare chromosomal transloca- tions occurring, and also by disturbing the normal virus–host balance, thereby enlarging the pool of EBV-infected cells in the body. It is clear that the virus is a necessary, but not sufficient element in the aetiology of endemic Burkitt lymphoma. Based on in vitro studies, EBV’s role in Burkitt pathogenesis is thought to be antiapoptotic (i.e. partially reducing the sensitivity to programme cell death that is a consequence of high MYC expression), thereby allowing MYC’s other action, as a driver of cell growth, to become dominant. Sporadic Burkitt lymphoma The sporadic form of the tumour occurs in children worldwide, but generally at a much lower incidence than endemic Burkitt lymphoma. The association of these tumours with EBV varies from 10 to 15% in the Western world, where the disease is 100-fold less frequent than in endemic areas, to more than 50% in some other countries where the incidence is intermediate between the two ex- tremes. The same restricted EBV gene expression is seen in virus- positive sporadic tumours as in the endemic disease and the role of EBV, when present, is thought to be similar. Symptoms and signs Unlike endemic Burkitt’s lymphoma, the sporadic form very rarely involves the jaws, and frequently presents in lymph nodes and within the abdomen. The clinical features depend on the location of the tumours.

 Diagnosis The tumour must be distinguished from other types of non-Hodgkin lymphoma by histological examination of biopsies. Sporadic Burkitt lymphoma has the same histologic features and carries the same type of MYC translocation as the endemic tumour, though more often with additional chromosomal aberrations.. Treatment The response to chemotherapy is not usually as good as in en- demic Burkitt lymphoma. Cyclophosphamide alone is

inadequate; combination therapy is required, and survival after relapse is uncommon. AIDS-associated Burkitt lymphoma Burkitt lymphoma appeared unexpectedly as a common tumour of HIV-positive adults during the early years of the AIDS epi- demic in the West. In many respects, the AIDS-associated tu- mour resembles the sporadic form seen in children, except that its incidence is much higher (even greater than the endemic dis- ease) and some 30–40% tumours are EBV-positive. The tumour tends to arise relatively soon in the course of AIDS, following the initial period of HIV-induced generalized persistent lymph- adenopathy and before the inset of profound T-cell impairment. This implies that HIV predisposes to Burkitt lymphomagenesis through stimulating germinal centre activity in the B-cell system (like hyperendemic malaria) rather than through its subse- quent destruction of the T-cell system. Accordingly, although highly active retroviral therapy (HAART) prevents T-cell im- pairment and progression to late-stage AIDS, the incidence of Burkitt lymphoma among HIV-positive cohorts has not fallen in the post-HAART era. Note that the same appears to be true of another AIDS-associated B-cell lymphoma, diffuse large B-cell lymphoma of germinal centre origin, whose incidence is also in- creased in HIV-infected individuals, albeit not as dramatically as Burkitt lymphoma; these tumours are less well studied as a histologically distinct group but appear to be EBV-positive in 30–50% cases; the virus' role in their pathogenesis remains to be determined. Lymphoproliferative disease/lymphoma of the immunocompromised T-cell impairment, whether congenital, induced by immunosup- pressive therapy, or caused by late-stage HIV infection, relaxes host control over EBV, leading to increased virus replication in the oral cavity, increased numbers of circulating virus-carrying B lymphocytes and a reappearance of EBV growth-transforming B-cell infections. The higher antigen load, arising as a consequence of relaxed T-cell surveillance, then induces higher levels of anti-EBV antibodies in serum. Such disturbance of the virus–host balance is initially asymptomatic but over time greatly increases the risk of EBV-associated lymphoproliferative disease. In primary immune deficiency states Besides the special case of XLP (described earlier), many other primary immune deficiency states affecting T-cell competence are now known and their genetic basis identified. Many of these are susceptible to the damaging effects of infection with a range of persistent viruses, including herpesviruses, that are normally con- tained by immune surveillance. EBV-driven lymphoproliferative lesions, essentially identical to those seen in posttransplant pa- tients (see next) are frequently reported in such cases, typically in those children who have survived long enough to acquire the virus. In transplant recipients Solid organ transplant recipients, who receive long-term immuno- suppressive drugs, have an up to 100-fold increase in their life- time risk of developing B lymphoproliferative disease/lymphoma compared with normal immunocompetent individuals. Most of these tumours occur within the first year of transplantation when immunosuppressive therapy is most intense. A similar picture is seen in haemopoietic stem cell transplant recipients who are at greatly increased risk in the first 6–12 months posttransplant before their immune system is fully re-populated. These early onset 'posttransplant' lymphoproliferative disorders are fre- quently oligoclonal and almost always EBV-driven, made up of EBV-positive B cells expressing the same range of eight growth-transforming latent proteins (6 EBNAs and 2 latent membrane proteins) just as seen in EBV-positive lymphoblastoid cell lines in culture. Such lesions arise through a failure of virus-specific immune T-cell surveillance and so occur with highest incidence in the most heavily immunosuppressed patients, particularly those (most often children) who were uninfected by the virus pretransplant and acquired it in the peritransplant period. The virus therefore appears to be both a necessary and sufficient cause of such lymphoproliferative disease. The lesions are refractory to conventional cytotoxic drug therapy and have attracted different treatment approaches. In the solid organ transplant setting, the initial treatment is to reduce the im- munosuppressive drug dose, with or

without aciclovir therapy, and in some patients a partial recovery of T-cell competence will be sufficient to clear the EBV-positive lesion without initiating graft-versus-host disease. In the stem cell transplant setting, treat- ment with Rituximab (monoclonal antibody to the B-cell surface antigen CD20) is now the preferred treatment option while the first effective treatment to be developed, adoptive transfer of in vitro- expanded EBV-specific T-cell preparations, is now reserved for Rituximab-resistant disease. Because solid organ transplant recipi- ents remain on low but continual immunosuppression, they also have an increased longer-term risk of lymphoma. Some of these late tumours resemble abovementioned lymphoproliferative lesions, whereas others are monoclonal B-cell lymphomas of more varied type, only some of which are EBV-positive; the role of the virus in this latter context is unclear. In people with HIV In the pre-HAART era, HIV-infected individuals who progressed to end-stage AIDS were at very high risk of an EBV-driven lymphoproliferative disease essentially similar to that seen in posttransplant patients. These lesions presented extranodally at many unusual sites, most commonly in the central nervous system (CNS) where they were frequently reported as ‘CNS

 lymphomas’. Disease progression is rapid, with a mean survival time from diagnosis of 3–4 months. Radio- and/or chemotherapy is disappointing because patients with late-stage HIV are often in poor general health. Hodgkin lymphoma There had long been a suspicion that EBV is involved in the pathogenesis of Hodgkin lymphoma, largely because the age and social class-dependence of this tumour’s incidence in the devel- oped Western world resembled that of infectious mononucle- osis; both diseases show a marked peak in young adulthood. Furthermore, it became clear that infectious mononucleosis carried with it a 4-fold increased risk of developing Hodgkin lymphoma over the next 10 years. Indeed, the increased risk was most marked within 2–3 years of the original attack of infectious mononucleosis. Hodgkin lymphoma is a monoclonal tumour of postgerminal centre B-cell origin, but unusual in that the malignant popu- lation (of Reed–Sternberg and mononuclear Hodgkin cells) typically accounts for only 1–2% of the tumour mass. Those cells are greatly outnumbered by a nonmalignant infiltrate, involving many cell types, whose different composition defines the histologically classified subtypes of the disease. The EBV genome is now known to be present in all the malignant cells in some 30–40% of Hodgkin tumours arising in the Western world. Surprisingly, it is the mixed cellularity and lymphocyte- depleted subtypes that show the highest levels of EBV-positivity (75–90%), whereas the nodular sclerosing subtype, which makes up the bulk of the young adult Hodgkin peak in Western soci- eties, is much less often EBV-positive. Thus, in the West, EBV is more strongly associated with Hodgkin lymphoma presenting in children and in older age patients. By contrast, in developing countries which lack the young adult peak, mixed cellularity and lymphocyte-depleted subtypes are the most common forms of the tumour independent of age, and up to 80% of all Hodgkin tumours are EBV genome-positive. Where present, EBV ex- presses EBNA1 and the two latent membrane proteins, LMPs 1 and 2, a pattern intermediate between the extremes seen in Burkitt lymphoma (EBNA1 only) and lymphoproliferative dis- ease of the immunocompromised (all 6 EBNAs and the two LMPs). EBV’s role in Hodgkin lymphomagenesis is not fully understood but, as in Burkitt lymphoma, its main influence is thought to be through protection against apoptosis rather than through directly driving cell growth. Undifferentiated Nasopharyngeal carcinoma This tumour is restricted to the postnasal space, where it arises from squamous epithelial cells. It has a distinct histology with heavy infiltration by nonmalignant T cells, and is thus some- times designated a lymphoepithelioma. The tumour is seen at low incidence worldwide but has a remarkably high incidence among people of southern Chinese origin, as well

as in the Inuit and related circumpolar peoples. In high-incidence areas, nasopharyngeal carcinoma is the most common cancer of men, and the second most common of women. The disease also occurs with intermediate incidence in Malays, Dyaks, Indonesians, Filipinos, and Vietnamese people, as well as in a belt stretching across North Africa, through Sudan, to the Kenyan highlands. The tumour usually occurs in middle or old age, but in North Africa it has bimodal age peaks, one involving young people up to 20 years old and a second, much later in life. Irrespective of geographical region, nasopharyngeal carcinoma cells always carry the EBV genome and express a subset of latent virus proteins, namely EBNA1, LMP1 (in some cases) and LMP2. Symptoms and signs Nasopharyngeal carcinoma causes nasal obstruction, discharge, or bleeding; deafness, tinnitus, or earache; and headache and ocular paresis from tumour spread to involve the cranial nerves. Patients may present with a single symptom caused locally by the tumour, or with several symptoms, and about one-third complain only of cervical lymph-node enlargement resulting from metastatic spread from an occult primary tumour. Direct spread from the primary tumour may involve the soft tissues, bone, parotid gland, buccal cavity, and oropharynx. The neoplasm may extend into the nasal fossae, the paranasal sinuses, or the orbit, and can invade the eustachian tube or the parapharyngeal space, where cranial nerves IX, X, XI, and XII can be involved. Invasion of the skull or cranial foramina may damage cranial nerves II, IV, V, and VI. Lymphatic spread causes enlarged cervical lymph nodes, and subsequently extends to the supraclavicular glands. If blood-borne metastases occur, they are most frequent in the bones, liver, and lungs, but may be in any organ. Differential diagnosis Nasopharyngeal carcinoma must be distinguished from other tumours of the nasal cavities, namely adenocarcinomas, sarcomas, malignant lymphomas, and rare malignancies such as chordoma, teratoma, and melanoma. Laboratory diagnosis The diagnosis of nasopharyngeal carcinoma is made histologically on a biopsy sample of the primary tumour or an enlarged cer- vical lymph node. Serum antibody titres to EBV antigens show a characteristic reaction pattern—IgG and IgA antibodies to VCA and diffuse early antigen are raised, with the titre correlating with the tumour burden. Uniquely, IgA antibodies to VCA and early antigen are also found in patients’ saliva. These antibody patterns often arise many months before the onset of detectable tumour growth, and have been used in a high-incidence area of China to screen the population for incipient cases and/or high risk individuals. Treatment Untreated nasopharyngeal carcinoma progresses inexorably to death, but it responds well to radiotherapy, which is the initial treatment of choice. In the earliest stages of the disease, radio- therapy gives 5-year survival rates of 50% or more, and of those surviving for 5 years, 70% remain permanently free of relapse.

 Treatment schedules combining radio- and chemotherapy are now giving some further improvement. However, the more ad- vanced stages of nasopharyngeal carcinoma still have correspond- ingly poor prognoses. Pathogenesis EBV is now widely accepted as an essential element in the caus- ation of nasopharyngeal carcinoma. Early studies showed that 100% of undifferentiated nasopharyngeal carcinomas world- wide are EBV-positive, and recent studies have also detected viral DNA in a subset of the more differentiated nasopharyngeal tumours. Thus all forms of nasopharyngeal carcinoma, irrespective of whether they originate in high- or low-incidence areas, may be associated with EBV. Both the tumour cells and the EBV gen- omes within them are clonal, indicating that the malignancy arises from a single malignantly transformed EBV-infected epithelial cell. Evidence from EBV latency genes expressed in nasopharyngeal carcinoma and premalignant lesions strongly suggest that viral gene products contribute to the abnormal epi- thelial proliferation, though the exact mechanism remains to be determined. Nonviral factors that predispose to tumour development in- clude racial and genetic

predispositions; many cases among southern Chinese people show a clear familial link, and certain HLA haplotypes are associated with the disease. Epidemiological studies also suggest that environmental cofactors associated with the Chinese way of life play a role. Two likely candidates are (1) traditional herbal medicines containing tumour-promoting phorbol ester-type substances, taken as snuff, and (2) trad- itional salted fish, which has been shown to contain carcinogenic nitrosamines. Salivary gland lymphoepithelioma These relatively rare tumours resemble nasopharyngeal carcinoma, both histologically and in their prevalence in circumpolar popu- lations. Although some are in reality nasopharyngeal cancers that have spread to the parotid gland from occult primaries, others are clearly of salivary gland origin. The EBV genome, which is clonal in all the malignant epithelial cells, is not found in any other type of salivary gland tumour. The association with EBV has not been suffi- ciently explored to assess its significance. Gastric carcinoma Some 8–10 10% of gastric carcinomas worldwide are EBV genome-positive. These include almost all of those rare gastric tumours with a lymphoepithelioma-type morphology, and a mi- nority of the more common gastric adenocarcinomas. There is increasing evidence from molecular studies that EBV-positive tumours constitute an aetiologically distinct subset of gastric carcinomas, with particular patterns of cellular gene expres- sion and of hypermethylated gene sets. Furthermore the viral genome is again found in monoclonal form in every cell of the EBV-positive tumour, providing another example of a tumour arising from a single EBV-infected cell. Viral antigen expression tends to be more limited than in nasopharyngeal carcinoma, with EBNA1 and in most cases LMP2 being detectable. As in the naso- pharyngeal tumour, EBV's contribution to carcinogenesis remains to be determined. T- and NK-cell lymphomas EBV is now also strongly linked to a particular type of T- or NK-cell lymphoma that typically presents as a granulomatous, bone-eroding, lesion. This usually occurs in the midline of the face, or as destructive lesions of the soft palate, or as multiple intranasal masses, hence the tumour's earlier description as le- thal midline granuloma. Such tumours are more common in men than women, and in Asian and South American populations than in people of European descent. However, all cases world- wide are EBV genome-positive and the tumour cells express a limited range of EBV latent antigens, namely EBNA1, LMP1 (in some cells), and LMP2. Other rare forms of the same lymphoma can also present in the skin, sometimes in association with hypersensitivity to mosquito bites. Yet others arise at various sites in individuals with a history of chronic active EBV infec- tion, where EBV has gained previous access to T and/or NK-cell lineages and has initiated oligoclonal proliferations of latently infected T or NK cells from which a monoclonal EBV-positive tumour arises (see 'Chronic active EBV infection', earlier). Occasionally, cases of EBV-positive NK-cell leukaemia may arise ab initio. Currently, all of these malignancies are extremely difficult to treat. Other EBV-associated conditions linked to profound immune impairment An unexpected consequence of profound T-cell impairment, first noted in children with progressive HIV infection but now also seen occasionally in heavily immunocompromised trans- plant recipients, is the appearance of smooth muscle tumours, leiomyomas, and leiomyosarcomas that are consistently EBV genome-positive. As yet, very little is known about how the virus accesses such an unusual cell type or how it contributes to its ma-lignant conversion. A second EBV-related disease of the immunocompromised state, again first noted in AIDS patients but also now seen occa- sionally in the posttransplant setting, is oral hairy leukoplakia. This is a non-malignant lesion that typically presents as painless white patches on the tongue or the lateral buccal mucosa. The le- sions are usually multiple, up to 3 cm in diameter, slightly raised, poorly demarcated, and have a hairy or corrugate surface. The differentiating squamous epithelial cells forming the outer layers of these lesions contain large amounts of actively replicating EBV, providing the only example of a disease resulting from productive

infection by the virus. Though the differentiation-dependence of this EBV replicative lesion superficially resembles that shown by

8.5.3 Epstein–Barr virus 763 papillomaviruses, there is a crucial difference in that EBV appears to be confined to the outer epithelial layers and there is no de- tectable infection, either latent or lytic, in the basal or immediate suprabasal layers. It is not clear whether oral hairy leukoplakia simply magnifies a process of EBV replication occurring sublim- inally in all virus carriers, or is an artefact of the heavily immuno- compromised state. In affected individuals, aciclovir treatment arrests virus replication and the leukoplakia lesions regress, but only for as long as the drug is continued. EBV and autoimmune conditions Recent years have seen a reawakening of interest in possible links between EBV and autoimmune disease, with the epidemiological findings strongest in the case of multiple sclerosis. Firstly a his- tory of infectious mononucleosis increases lifetime risk of mul- tiple sclerosis by 2 to 3-fold. Secondly almost all (>99%) multiple sclerosis patients are EBV-seropositive (i.e. already EBV-infected) at the time of disease presentation compared to 90–95% matched healthy controls. Thirdly, a large prospective study found that, of 305 military recruits who subsequently developed multiple sclerosis, only 10 were EBV-seronegative at recruitment and all 10 seroconverted 2–6 years before disease presentation. Whether such evidence truly reflects a role for EBV in the pathogenesis of multiple sclerosis, and what that role might be, remain hotly de- bated questions. Prospects for a prophylactic EBV vaccine Given the wide range of nonmalignant and malignant diseases with which EBV is aetiologically linked, a vaccine able to protect against infection would have an enormous global impact. Efforts in that direction have re-doubled following the development of successful vaccine against the oncogenic human papillomavirus types 16 and 18. Whether the papillomavirus vaccine strategy, based on the induction of virus-neutralizing antibodies, will be effective in the context of EBV, a herpesvirus with a more com- plex biology and different transmission route, remains unclear. Human trials of a new vaccine, using a multimeric form of the major EBV envelope glycoprotein gp350 as immunogen, will soon begin to resolve that question. FURTHER READING Ascherio A, Munger KL (2010). Epstein–Barr virus infection and multiple sclerosis: a review. J Neuroimmune Pharmacol, 5,
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