

8. Viral cancers

Viruses linked to cancers in humans are the Epstein-Barr virus (EBV), associated with lymphomas and nasopharyngeal cancer, hepatitis B virus (HBV) and hepatitis C virus (HCV), both associated with cancer of the liver, human papillomaviruses (HPV), associated with cancer of the cervix, human T lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2), associated with adult T-cell leukemia and with hairy-cell leukemia, respectively, and human herpesvirus 8 (HHV-8), associated with Kaposi sarcoma. No vaccine exists against these viruses except HBV. This chapter will describe vaccines under development against EBV, HCV and HPV.

8.1. Epstein-Barr virus

In the 1950s, Denis Burkitt described the existence of B-cell lymphomas in 2–14 year-old African children from malaria endemic areas. In 1964, continuous B-lymphocyte cell lines derived from these tumors were found by Epstein and Barr to spontaneously release a herpesvirus. It was Gertrud and Werner Henle who demonstrated that the Epstein-Barr virus (EBV) is ubiquitous in the human population where it is usually the cause of infections that are not apparent though it may cause infectious mononucleosis. The more severe, albeit rare, result of EBV infection is malignant transformation and cancer development in various forms, including Burkitt's lymphoma and nasopharyngeal carcinoma, one of the most common cancers in China.

8.1.1. Disease burden

The primary site of Epstein-Barr virus (EBV) infection is the oropharyngeal cavity. Children and teenagers are commonly afflicted usually after oral contact, hence the name "kissing disease". Based on serology, about 95% of the world adult population has been infected with EBV and, following primary infection, remains lifelong carriers of the virus. In developed countries, exposure to EBV occurs relatively late: only 50–70% of adolescents and young adults are EBV seropositive. About 30% of the seronegative group will develop infectious mononucleosis as a result of primary EBV infection. The disease is characterized by fever, sore throat, generalized lymphadenopathy, splenomegaly, intense asthenia, hyper-lymphocytosis (>50%) with atypical lymphocytes and elevated transaminase levels. In developing countries, EBV antibodies are acquired early in life and the disease is mostly asymptomatic.

EBV is associated with Burkitt's B-cell lymphoma and nasopharyngeal carcinoma. Burkitt's lymphoma (BL) is a malignant form of tumor associated with EBV that is endemic to central parts of Africa and New Guinea with an annual incidence of 6–7 cases per 100 000 and a peak incidence at 6 or 7 years of age. The epidemiological involvement of EBV in Burkitt's lymphoma is based on the recognition of the EBV viral genome in tumor cells, associated with an elevated antibody titre against EBV viral capsid antigen (VCA). The highest prevalence of BL is found in the "lymphoma belt," a region that extends from West to East Africa between the 10th degree north and 10th degree south of the equator and continues south down the Eastern coast of Africa. This area is characterized by high temperature and humidity, which is probably the reason why an association of malaria with BL was suspected at one time. In African countries such as Uganda, in the lymphoma belt, the association of BL with EBV is very strong (97%), whereas it is weaker elsewhere (85% in Algeria; only 10–15% in France and the USA).

Nasopharyngeal cancer (NPC) incidence rates are less than 1 per 100 000 in most populations, except in populations in southern China, where an annual incidence of more than 20 cases per 100 000 is reported. Isolated northern populations such as Eskimos and Greenlanders also show high incidence. There is a moderate incidence in North Africa, Israel, Kuwait, the Sudan and parts of Kenya and Uganda. Men are twice as likely to develop NPC as women. The rate of incidence generally increases from ages 20 to around 50. In the USA, Chinese-Americans comprise the majority of NPC patients, together with workers exposed to fumes, smoke and chemicals, implying a role for chemical carcinogenesis. Studies related to nutrition and diet have shown an association between eating highly salted foods and NPC. Vitamin C deficiency at a young age also may be a contributing factor. Finally, a study of HLA haplotypes revealed a genetically distinct subpopulation in southern China, with an increased frequency of haplotype A-2/B-Sin-2 which may account for the higher disease incidence in the area.

Recent studies have shown that EBV also is associated with B-cell malignancies such as Hodgkin's lymphoma (HL) and lymphoproliferative disease in immunosuppressed patients, as well as with some T-cell lymphomas and other epithelial tumors such as gastric cancers. These tumors are characterized by the presence of multiple extrachromosomal copies of the viral genome in tumor cells and the expression of part of the EBV genome.

8.1.2. Virology

EBV, together with HHV-8 (Kaposi sarcoma-associated virus), belongs to the genus *Lymphocryptovirus*, in the subfamily *Gammaherpesvirinae*, family *Herpesviridae*. These are complex enveloped DNA viruses, which multiply in the nucleus of the host cell (*see* 5.3). EBV infects resting human B-lymphocytes and epithelial cells, multiplies in the latter and establishes latent infection in memory B-lymphocytes. Thus, infected individuals may produce virions, carry virus-specific CTLs, produce EBV-specific antibody, and yet harbor latently infected memory B-cells. These maintain the latent EBV genome as an episome that expresses only part of its genetic information, including EBV nuclear antigens EBNA-1 (a latent DNA replication factor), EBNA-2 (a transcriptional activator) and EBNA-3A and -3C (involved in the establishment of latency), together with integral membrane proteins LMP-1 and LMP-2 which play major roles in maintenance of latency and escape from the immune response of the host. Latently infected cells do not produce the B7 coactivator receptor and, therefore, are not killed by CTLs. When peripheral blood from an infected individual is cultured, latently infected B-cells begin to replicate and yield immortalized progeny lymphoblasts that can be indefinitely propagated in the laboratory.

The major EBV external surface glycoprotein is a 350 kD antigen, gp350/220, which binds the CD21 receptor on B-cells. Another envelope glycoprotein, gp42, is responsible for the fusion between the virus envelope and the host cell membrane. The EBV genome, a 172 kbp linear double-stranded DNA molecule, becomes circular for replication and latency. Viral capsid antigens (VCA) are late gene products.

8.1.3. Vaccines

The development of an EBV vaccine could protect individuals against primary infection and hence presumably reduce the burden of EBV-associated cancers.

The principal target of EBV neutralizing antibodies is the major virus surface glycoprotein gp350/220. Several vaccine candidates based on gp350/220 have been developed. Live recombinant vaccinia virus vectors have been used to express the gp350/220 antigen and were found to confer protection in primates and elicit antibodies in EBV-negative Chinese infants.

Soluble recombinant gp350/220 produced in CHO cells was found to be safe in humans but needed strong adjuvants to elicit acceptable immunogenicity (co-development by MedImmune, GSK and Henogen). Phase II clinical trials of this candidate vaccine are under way.

Clinical trials of an EBNA-3A peptide are being conducted in Australia.

8.2. Hepatitis C

The majority of the worldwide hepatitis burden, with subsequent chronic hepatitis, cirrhosis and liver cancer is due to hepatitis virus B (HBV), which kills 4000 to 5000 Americans each year, and about 1.2 million people worldwide. Approximately 350 million people have chronic hepatitis B infection, with endemic areas primarily in Africa and Asia. Fortunately, the global burden of hepatitis B should eventually decrease as affordable recombinant subunit vaccines based on the surface antigen of the virus (HBsAg) and effective control strategies are deployed to control the disease on a global basis. Infants in developing countries begin their HBV immunization at birth; this has resulted in dramatic reductions in virus transmission in high-risk populations, and in decrease in incidence of liver cancer, as seen in China (Province of Taiwan).

Other viral hepatitis, initially regrouped under the designation “Non A-non B” hepatitis and against which there is still no vaccine, include hepatitis C and hepatitis E. The search for a possible “non A-E” virus which would be responsible for the 4% acute cases of hepatitis of undiagnosed origin led to the successive identification of the HGV/GBV-C, TTV and SEN-V viruses, none of which appear to be the right candidate.

8.2.1. Disease burden

Hepatitis C has been compared to a “viral time bomb”. WHO estimates that about 180 million people, some 3% of the world's population, are infected with hepatitis C virus (HCV), 130 million of whom are chronic HCV carriers at risk of developing liver cirrhosis and/or liver cancer. It is estimated that three to four million persons are newly infected each year, 70% of whom will develop chronic hepatitis. HCV is responsible for 50–76% of all liver cancer cases, and two thirds of all liver transplants in the developed world. Disease prevalence is low (< 1%) in Australia, Canada and northern Europe, about 1% in countries of medium endemicity such as the USA and most of Europe, and high (>2%) in many countries in Africa, Latin America and Central and South-Eastern Asia. In these countries, prevalence figures between 5% and 10% are frequently reported. The extremely high seroprevalence of HCV in the Nile delta of Egypt was found to increase with age from 19% in those 10–19 years of age to about 60% in 30 year-old persons, and is thought to be the major cause of the high prevalence of liver cirrhosis in the country.

Current estimates in the USA are that 3.9 million Americans are chronically infected with HCV, with prevalence rates as high as 8–10% in African Americans. Haemodialysis patients, haemophiliacs, drug addicts and people transfused with blood before 1990 are particularly affected by the disease. Injectable drug use remains the main route of transmission, accounting for nearly 90% of new HCV infections. Sexual transmission is thought to be relatively infrequent.

Mother-to-child HCV transmission has been widely documented. The risk of perinatal infection ranges from 3–15% in different populations. Transmission is believed to occur in utero, as a consequence of a high viral load in the mother. However, correlates of transmission remain to be defined and targeted studies are needed to provide adequate counseling to HCV-infected pregnant women and to identify possible preventive measures.

HCV infection is asymptomatic or paucisymptomatic in 90% of cases. In contrast with viral hepatitis A or B, jaundice is relatively rare, and fulminant hepatitis forms are rarely observed. In 50–80% of adult cases, the immune system is nevertheless unable to eliminate the virus and the disease becomes chronic, with persistent asthenia and vascularitis, porphyria cutanea, glomerulonephritis and others. The patients usually show elevated transaminase levels and mixed cryoglobulinemia. Chronic hepatitis C disease is the first cause of liver transplantation in developed countries. Furthermore, about 20–50% of chronically infected persons will eventually develop cirrhosis or cancer of the liver. Incidence rates of hepatocellular carcinoma among patients with HCV-related cirrhosis is highest in Japan. It has been estimated that only about 50% of HCV-infected persons are diagnosed in most developed countries and that two-thirds of them need to undergo antiviral treatment.

8.2.2. Virology

HCV belongs to the genus Hepacivirus in the family *Flaviviridae*. There are 6 HCV genotypes and more than 100 subtypes. In addition, HCV, very much like HIV, is characterized by the continuous emergence of virus variants, thus making a moving target for vaccine design. Like other flaviviruses, HCV is an enveloped virus with an icosahedral capsid that contains a 9.6 kb-long, single-stranded, positive sense genomic RNA. The virus does not grow in cell culture; this made its initial identification in 1989 a molecular biology *tour de force* and does not facilitate the selection of attenuation mutants or the titration of neutralizing antibodies. Its envelope contains two glycoproteins, E1 and E2, which form heterodimers at the surface of the virion. The genomic RNA is translated into a viral polyprotein which is cleaved by cellular proteases to generate the capsid protein (C), the two glycoproteins E1 and E2, a small protein the role of which is unclear (p7), viral proteases NS2 and NS3 and nonstructural proteins NS4A and 4B and NS5A and 5B, which are required for viral RNA replication. The putative HCV receptor has recently been identified as protein CD81.

8.2.3. Vaccine development

The development of an HCV vaccine is an obvious necessity as an overall 50% of treated patients do not experience significant long-term benefits from the current pegylated interferon and ribavirin-based combination therapy. Such a development, however, meets with many obstacles. Chimpanzees remain the only animal model for HCV infection, but they are an endangered species and difficult to work with because of high costs and other restrictions. Even though HCV infection generates antibodies, none of these seem capable of resolving the infection. One reason might be that the virus does not appear to circulate as free virions but is always found in association with lipoprotein particles or immune complexes. Recovery from acute hepatitis is typically associated with broad and early class II-CD4⁺ responses and class I-CD8⁺ responses to HCV. A vaccine, to be successful, will presumably need to elicit strong CTL and T helper cell responses. It also will have to face high variability of the virus favouring immune evasion.

No vaccine is yet available. Several vaccine approaches, essentially therapeutic, are currently in development.

- Native heterodimer complexes comprising both envelope glycoproteins E1 and E2 have been produced in CHO cells and used as a subunit vaccine added with the MF59 adjuvant (Chiron). The vaccine elicited high titre antibodies and CD4⁺ T-cell responses and provided nonsterile protection against challenge in 50% of the vaccinated chimpanzees. Phase I trials of this vaccine are in progress.

- A vaccine candidate based on recombinant E1 in alum, developed by Innogenetics in Europe, has reached Phase II trials in non-responders to interferon treatment. Results showed that it is well tolerated and seems to slow down the progression of liver fibrosis, but no changes in plasma HCV loads were detected, despite decreased antigen levels in the liver and strong antibody and cellular responses to E1. An additional Phase II randomized study should be completed in 2005.
- VLPs (HCV-LPs) were produced in insect cells using a recombinant baculovirus expressing the cDNA of the HCV structural proteins C, E1 and E2. This approach is attractive because particulate structures are more immunogenic than soluble proteins. HCV-LPs resemble the putative HCV virions and induce strong HCV-specific immune responses in mice and baboons, including antibodies to HCV structural proteins and IFN- γ CD4+ and CD8+ T-cell responses. The immunogenicity of the HCV-LPs was only marginally enhanced by the addition of CpG oligonucleotides or the ASO1 formulation as adjuvants.
- An immunostimulatory complex formulation (ISCOMATRIX) with the HCV core antigen has been studied by CSL in Phase II trials in Australia, in collaboration with Chiron.
- HLA-A2-restricted core epitope peptides formulated with influenza virosomes as carriers are being developed by Berna/Pevion in Switzerland for both therapeutic and prophylactic vaccine strategies. This formulation is in preclinical studies. To improve the induction of T-cell immunity, the amino acid sequence of the peptides was modified so as to increase their affinity for the HLA molecule (epitope enhancement)
- Several other vaccine projects are at an early preclinical stage, including HCVACC and Innogenetics in Europe and GenPhar, Epimmune, and Merix in the USA.
- Two Chinese teams also are making significant progress in HCV vaccine research: Fudan University and the National Taiwan University, Taipei, China (Province of Taiwan).
- Finally, an MVA-based live recombinant vaccine expressing three NS protein genes should reach the clinics by the end of 2005 (BioMérieux, France).

8.3. Human papillomavirus

8.3.1. Disease burden

Human papillomavirus (HPV) causes cervical cancer, the second biggest cause of female cancer mortality worldwide with an estimated 240 000 deaths yearly. The prevalence of genital HPV infection in the world is around 440 million, and that of clinical infections about 160 million. Genital HPV infection is extremely common and most often remains subclinical, but a proportion of the infected individuals with low-risk HPV types such as HPV-6 or HPV-11 will develop genital warts, whereas a subset of women with high-risk HPVs such as HPV-16 or HPV-18 will develop preneoplastic lesions of cervical intraepithelial neoplasia (CIN). Low-grade cervical dysplasias are common and most regress spontaneously. In contrast, the minority of lesions that progress to high-grade dysplasias tend to persist and/or progress to carcinomas in situ before becoming invasive cancers. The majority of adenocarcinomas of the cervix and of squamous cell cancers (SCC) of the vulva, vagina, penis and anus are caused by HPV-16 (57% of cases) and HPV-18 (14% of cases), the remaining 30% being due to less prevalent high-risk HPV types (HPV-31, -33, -35, -39, -45, -51, -66, etc.).

About 490 000 cases of cervical cancer are reported each year, nearly 80% of which from developing countries, where neither population-based routine screening (Papanicolaou smear test) nor optimal treatment is available; estimated figures are 265 000 cases in South-East Asia, 79 000 in Africa, and 72 000 in Latin America. The highest yearly incidence of cancer of the cervix is found in some countries of Central and South America (e.g. Haiti), southern Africa (e.g. Tanzania), and Asia (India). Epidemiological studies in the USA have reported that 75% of the 15–50 year-old population is infected with genital HPV over their lifetime, 60% with transient infection (antibodies), 10% with persistent infection (detection of DNA), 4% with mild cytological signs, and 1% with clinical lesions.

HPV infection also can lead to papillomas in the oral cavity and in the upper respiratory tract. In HIV-infected individuals, HPV infection appears to cause extensive warts and severe and rapidly progressing disease.

8.3.2. Virology

HPV belongs to the family *Papovaviridae*. These are small nonenveloped icosahedral viruses with an 8 kbp-long double-stranded circular DNA genome. More than 100 different HPV types have been identified on the basis of genomic nucleotide sequence homology, some 40 of which can infect the ano-genital mucosa. The papillomavirus genome comprises early and late genes that encode early proteins E1–E7 and late proteins L1–L2. The early proteins are nonstructural proteins involved in replication and transcription of the genome (E1–E5) or in host cell tumoral transformation (E6 and E7), whereas L1 and L2 are the structural capsid proteins of the virion. The low-grade cervical dysplasias correspond to productively infected cells that actively shed virus, whereas high-grade dysplasias and cancers do not produce virions: viral gene expression in these cells is limited to the E6 and E7 oncogenes that are transcribed from randomly integrated viral DNA. The E7 protein is thought to induce cell proliferation and disrupt the cell cycle regulation by inactivation of the Rb family proteins, whereas E6 blocks cell apoptosis by directing the p53 tumor suppressor protein to the proteasome.

8.3.3. Vaccines

Prophylactic HPV vaccine candidates are based on recombinant capsid protein L1 and aim to elicit neutralizing antiviral antibodies to protect against infection, while therapeutic vaccine candidates are based on viral oncogenic proteins E6 and E7, and aim to induce cell-mediated immune responses to eliminate the transformed tumor cells.

8.3.3.1. Preventive vaccines

The most advanced and promising approach for a prophylactic vaccine involves the use of noninfectious virus-like particles (VLPs) which self-assemble spontaneously from pentamers of the L1 capsid protein. These VLPs can be produced in baculovirus-infected insect cells or in yeast. They induce high titres of virus-neutralizing antibodies even in the absence of an adjuvant. In preclinical studies, vaccination of animals resulted in excellent protection from homologous virus challenge, and passive transfer of antibodies from the vaccinated animals also conferred protection.

Two prophylactic vaccine candidates are at the level of Phase III clinical evaluation. GSK is focusing on a bivalent HPV-16,-18 VLP vaccine candidate, based on baculovirus technology, and Merck is developing a tetravalent vaccine based on VLPs from HPV-6, -11, -16, and -18, using yeast-recombinant technology. A pilot efficacy trial of the monovalent HPV-16 vaccine showed that after 17 months follow-up, none of the 768 vaccinated young women acquired persistent HPV infection, whereas 41 of the 765 placebo recipients became

persistently infected with HPV-16, 5 of whom were at CIN-1 grade and 4 at CIN-2/3 grade. A comparable study by GSK has reported similar results with 100% efficacy against HPV-16 and -18 persistent infections. A Phase III multicentre clinical trial of the tetravalent vaccine has been launched by Merck to define efficacy against HPV-6/11-related genital warts, HPV-6/11/16/18-related CIN-1 lesions, and HPV-16/18-related CIN 2/3 lesions. The VLP vaccines are expected to be on the market by 2007.

A recombinant attenuated *Salmonella typhimurium* that expresses a HPV-16 L1 capsid gene whose codon usage was optimized to fit with the most frequently used codons in *Salmonella* was engineered at the University of Lausanne, Switzerland, and found to induce high titres of HPV neutralizing antibodies in mice after a single nasal or oral immunization with live bacteria. Testing of the vaccine in human volunteers is at the planning stage.

8.3.3.2. Therapeutic vaccines

Therapeutic vaccine candidates also have been developed, several of which have undergone Phase I/II clinical evaluation.

- A live recombinant vaccinia virus expressing modified versions of the E6 and E7 genes from HPV-16 and -18 (TA-HPV) has been tested by Xenova in two open-label Phase IIa trials in women with high grade vulvar intraepithelial neoplasias (VIN). A single immunization with TA-HPV induced at least 50% reduction in lesion size in 44% of the vaccinated patients. An additional study evaluated the combination of TA-HPV with TA-CIN, a recombinant fusion protein made up of the L2, E6 and E7 proteins of HPV-16, produced in *E. coli*. Three immunizations with TA-CIN followed by a single immunization with TA-HPV resulted in 23% of the patients experiencing a >50% reduction in VIN lesion size.
- Another recombinant bacterial fusion protein of HPV-16 E6 and E7 formulated with the ISCOMATRIX adjuvant has been made by CSL and shown to elicit good immune responses in a Phase I study.
- Transgene is developing a MVA-based vaccine that expresses modified HPV-16 E6 and E7 proteins, as well as the IL-2 cytokine. The vaccine is aimed at treating cervical as well as ano-genital dysplasias. In an initial Phase II clinical trial in women with CIN2/3, 43% of the patients receiving the highest dose of the vaccine showed clinical improvement within 6 weeks. A second trial is now under way, using this high dose in 18 women with CIN2/3 who will be followed for a 6-month period.
- Stressgen has conducted a number of Phase II clinical trials with a fusion protein made of E7 and heat shock protein (HspE7). In a Phase II study on 133 patients with anal dysplasia, there was no difference in adjudicated pathological response between vaccine and placebo recipients, although a significant effect was noted by the treating physician in “global assessment” scoring. The HspE7 vaccine was also shown to induce a 40% response rate within 8 weeks in a trial in 21 women with high grade dysplasia. Elucidation of the full extent and duration of the clinical benefit will require additional long-term follow-up.
- Finally, Zycos Inc. (now MGI Pharma) is developing a DNA plasmid-based therapeutic vaccine which, in a Phase II study, provided resolution of 43% pre-cancerous lesions caused by HPV in vaccinated women as compared to 23% in placebo recipients.

Medigene, in partnership with Schering AG, has developed a “chimeric” VLP vaccine (CVLP) using L1 or L2 recombinant proteins fused to modified E7 or E2 oncogenic

antigens. This technology allows the combination of both prophylactic and therapeutic components in the same immunogen. The safety of these vaccine candidates has been successfully tested by Medigene but their reported immunogenicity and efficacy were unsatisfactory.