Viruses: Eight Listings

Approximately 12% of cancers worldwide are caused by viruses (Parkin 2006, Carrillo-Infante *et al.* 2007). Hepatitis B virus (HBV), hepatitis C virus (HCV), and some human papillomaviruses (HPV) of the genital-mucosal type were first listed separately in the *Eleventh Report on Carcinogens* (2004) as *known to be human carcinogens*. The following five viruses have been added to the *Fourteenth Report on Carcinogens* as *known to be human carcinogens*: Epstein-Barr virus (EBV), human immunodeficiency virus type 1 (HIV-1), human T-cell lymphotropic virus type 1 (HTLV-1), Kaposi sarcoma–associated herpesvirus (KSHV), and Merkel cell polyomavirus (MCV). The profiles for all eight of these viruses (or virus families) follow this introduction.

For some viruses, in addition to epidemiological studies, evidence from clinical and molecular studies of tissues from infected individuals played a key role in the determination of sufficient evidence for carcinogenicity in humans. Several factors considered in the evaluation of cancer causality for each virus are discussed in the introductions to the Report on Carcinogens monographs on EBV, HIV-1, HTLV-1, KSHV, and MCV (NTP 2016a–e). The following are examples of key factors considered: (1) whether the virus is detected in the tumor tissue of most patients with the specific type of cancer, (2) whether the virus codes for production of proteins that are involved in tumor development, and (3) whether there is evidence to suggest that almost all the cells of a tumor contain viral DNA derived from a single ancestral virus particle (i.e., the viral DNA is monoclonal), suggesting that infection preceded tumor growth. However, not all of these lines of evidence are required in order to support the conclusion that a virus is carcinogenic.

Viruses may lead to cancer by direct mechanisms (such as expression of viral oncogenes), indirect mechanisms (such as immunosuppression), or both. Many viruses are more likely to cause cancer in patients with impaired immune systems. For example, HIV-1 infection impairs the body's immune system so that it cannot adequately suppress or destroy other cancer-causing viruses (including EBV, HBV, HCV, HPV, KSHV, and possibly MCV) or cells infected with these viruses, resulting in an increased risk of cancers specific to these non-HIV-1 viruses. Another way in which immunosuppression may lead to cancer is by facilitating the establishment of persistent latent viral infections and uncontrolled growth of the infected cells, leading to formation of tumors.

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Epstein-Barr Virus

CAS No.: none assigned

Known to be a human carcinogen

Also known as EBV or human herpesvirus 4 (HHV-4)

Carcinogenicity

Epstein-Barr virus (EBV) is *known to be a human carcinogen* based on sufficient evidence from studies in humans. This conclusion is based on evidence from epidemiological, clinical, and molecular studies, which show that Epstein-Barr virus causes endemic Burkitt lymphoma, Hodgkin lymphoma, immune-suppression-related non-Hodgkin lymphoma, nasal-type extranodal natural killer (NK)/T-cell lymphoma, nasopharyngeal carcinoma, and some forms of stomach cancer. There is also limited evidence for an association between EBV and sporadic Burkitt lymphoma. Results from clinical and mechanistic studies indicate that in EBV-infected B lymphocytes (a type of immune cell), EBV viral proteins are produced while the virus is latent (i.e., while the virus persists in cells without destroying them). These proteins (including latent membrane protein 1 [LMP-1] and Epstein-Barr nuclear antigens [EBNA-1, 2, 3A, and 3C]) enable the infected B lymphocytes to survive and proliferate indefinitely, leading to cancer in some cases. The specific protein involved in carcinogenicity may vary with the cancer end point. Furthermore, in most EBV-related cancers, a large proportion of tumor cells contain viral DNA that is derived from a single ancestral virus particle (i.e., monoclonal), suggesting that the viral infection preceded the tumor.

Cancer Studies in Humans

EBV was the first cancer-causing (oncogenic) virus to be discovered in humans; its association with Burkitt lymphoma was recognized over 50 years ago (Epstein *et al.* 1964). Burkitt lymphoma is a cancer of the immune cells with three subtypes; two of these, the endemic variant, which occurs primarily in children in equatorial Africa and Papua, New Guinea, and the sporadic variant, which is found throughout the world, are discussed here. Since the 1960s, numerous studies have explored the relationship of EBV to various other types of cancer, primarily other types of lymphoma — Hodgkin lymphoma and rare types of non-Hodgkin lymphoma (those related to immunosuppression or developing in NK or T cells in the nasal cavity) — and tumors arising from epithelial tissue, such as nasopharyngeal cancer and stomach cancer.

Burkitt Lymphoma

Evidence for an association between EBV infection and endemic Burkitt lymphoma is based on consistent findings of increased risk in several case-control and cohort studies and the presence of an exposure-response relationship with viral infection. Twelve case-control studies and a cohort study found statistically significant positive relationships between EBV infection and endemic Burkitt lymphoma, with risk increased by 3-fold to 52-fold. Moreover, several of the case-control studies and the cohort study found increasing risk of endemic Burkitt lymphoma with increasing viral load (primarily by measurement of antibodies to the viral protein VCA, viral capsid antigen) (Henle *et al.* 1969, 1971, Carpenter *et al.* 2008, Mutalima *et al.* 2008). Epidemiological evidence for an association between EBV infection and sporadic Burkitt lymphoma is somewhat weaker: positive associations were found in four of five case-control studies, one of which was statistically significant, and no studies evaluated exposure-response relationships. These studies had limited power to detect an effect because of the small numbers of EBV-infected case and control subjects.

These findings are supported by studies of human tumor tissue. EBV has been found in approximately 95% of endemic Burkitt lymphomas (Thompson and Kurzrock 2004) and in approximately 20% of sporadic Burkitt lymphomas (IARC 1997). The key event in the development of Burkitt lymphoma is a chromosomal translocation of *c-myc* (a regulatory gene) to the locus of an immunoglobulin gene promoter, which results in unregulated and persistent expression of *c-myc* RNA and proteins, which in turn regulate other genes, leading to proliferation and growth of B lymphocytes. In addition, Burkitt lymphomas infected with EBV produce a protein (EBNA-1) that plays a role in the prevention of apoptosis (programmed cell death) and enables cell survival (Lu *et al.* 2011, Kennedy *et al.* 2003).

Individuals infected with malaria in addition to EBV have been shown to have a higher risk of Burkitt lymphoma than individuals infected only with EBV. Malarial infection increases the numbers of mature B lymphocytes, many of which have chromosome damage, including EBV-induced chromosomal translocations (Robbiani *et al*. 2015). This reduces the ability of T lymphocytes to recognize and destroy infected cells and thus increases the viral load of EBV in infected individuals (Moormann *et al.* 2009).

Hodgkin Lymphoma

Evidence for an association between EBV infection and Hodgkin lymphoma is based on consistent findings of statistically significant increased risk in numerous case-control and cohort studies. The strongest evidence for an association comes from the collective findings of over 25 case-control studies, which found that Hodgkin lymphoma patients were generally 4 to 19 times more likely to have a high viral load (as measured by antibodies against viral proteins or, to a lesser extent, EBV DNA) than were individuals without Hodgkin lymphoma (NTP 2016). In a cohort study, individuals with higher levels of various types of EBV antibodies were approximately 3 to 4 times (depending on the specific antibody) more likely to develop Hodgkin lymphoma than those with lower levels of antibodies (IARC 1997, 2012). Most of the 11 case-control studies and 7 cohort studies using infectious mononucleosis as a surrogate for EBV infection also found statistically significant increased risks for Hodgkin lymphoma, though lower (generally between 1.3 and 3.0) than the risks found in the case-control studies with direct measurements of EBV infection (IARC 1997, 2012, Linabery *et al.* 2014). A few studies did not find a positive association, and not all studies found significantly increased risk. However, the strength of the database (i.e., the large number of studies using different study designs, conducted in different geographical locations, and measuring EBV by different methods) and the consistent findings of a relatively large, statistically significant increased risk, provide evidence that EBV infection causes Hodgkin lymphoma.

These findings are supported by molecular studies of human tissue or cell lines. Some forms of Hodgkin lymphoma are associated with monoclonal EBV infection. Hodgkin Reed-Sternberg cells are cancerous lymphocytes that contain EBV DNA and produce the viral proteins LMP-1 and -2A. These proteins promote growth and survival of the cancer cells by enhancing several molecular pathways (such as the NF-κB, JAK/STAT, MAP kinase, and PI3-kinase/AKT pathways), resulting in a release of cytokines (proteins that affect communication and interaction between cells) that causes a local inflammatory response (IARC 1997, 2012, Mohamed *et al.* 2014).

Immune-Suppression-Related Non-Hodgkin Lymphoma

Evidence that immune-suppression-related non-Hodgkin lymphoma is associated with EBV infection comes primarily from case series, a nested case-control study, and clinical studies showing the presence of EBV in these tumors. EBV has been found in lymphoma patients with various types of immunosuppression, including all cases of lymphoma in patients with congenital immune deficiency, almost all cases of non-Hodgkin lymphoma of the central nervous system in patients with HIV-1, and 50% of organ-transplant recipients with post-transplant lymphoproliferative disease (IARC 1997, 2012). In one study, EBV DNA was detected in the blood plasma of all patients with post-transplant lymphoproliferative disease but not in 35 healthy control subjects (Lei *et al.* 2000). A small nested case-control study found an association (although not statistically significant) between EBV antibodies and an increased risk of non-Hodgkin lymphoma in HIV-1-positive patients participating in a trial of antiretroviral therapy (Newton *et al.* 2006). Other studies in humans have shown that the EBV DNA in the tumors is monoclonal and produces oncogenic viral proteins (LMP-1, -2A, and -2B and EBNAs) that promote cell division, cell survival, and transformation into cancer cells. Finally, treatment of immune-suppressed patients with T cells sensitized to EBV has been shown to reduce viral load and reduce or protect against the formation of this tumor (Taylor *et al.* 2005, Vegso *et al.* 2011, IARC 2012).

Extranodal NK/T Cell Lymphoma, Nasal Type

Evidence that this type of lymphoma is associated with EBV infection comes primarily from more than a dozen case-series studies, with over 400 cases (IARC 1997, Barrionuevo *et al.* 2007, He *et al.* 2007), in which EBV was detected in 100% of the tumors. Nasal-type extranodal NK/T-cell lymphoma most commonly develops in NK cells, but can also occur in cytotoxic T cells. Two studies found EBV DNA in the blood plasma or CD3+ (T) cells from patients with this type of lymphoma, but not from healthy control subjects (Lei *et al.* 2000, Suwiwat *et al.* 2007). Other studies in humans have shown that the EBV DNA in the individual tumors is monoclonal and produces several viral proteins (LMP-1 and -2A and EBNA-1) involved in tumor development (IARC 2012).

Nasopharyngeal Carcinoma

Evidence for an association between EBV infection and nasopharyngeal carcinoma is based on consistent findings of highly increased risk in numerous case-control and cohort studies conducted largely in Southeast Asia, and also in Europe, North Africa, and the United States. The strongest evidence comes from the collective findings of 11 case-control studies, all of which reported statistically significant increased risks for this type of cancer, with most risk estimates ranging from 21 to 138 in studies measuring EBV antibody, and 41 to 820 in the studies measuring EBV DNA or DNase (an enzyme that degrades DNA). Increased risks were also found in two cohort studies (totaling 168 cases with up to 16 years of follow-up) but not in two small nested case-control studies, one among Alaska Natives in the United States (where nasopharyngeal cancer is rare) and the other among the general U.S. population. Other studies reported significantly higher mean EBV viral loads in case subjects than in control subjects (IARC 1997, 2012, NTP 2016).

These findings are supported by data from molecular studies. Monoclonal forms of EBV DNA are found in precancerous lesions and with 98% of non-keratinizing nasopharyngeal carcinomas, indicating that viral infection is an early event in cancer progression (Pathmanathan *et al.* 1995, Liu *et al.* 2011, Tsang *et al.* 2014, Tsao *et al.* 2015), and EBV has been shown to produce viral proteins (LMP-1 and -2A and EBNA-1) involved in carcinogenesis (Raab-Traub 2002).

Stomach (Gastric) Cancer

Epidemiological, case-series, clinical, and molecular studies provide evidence for an association between EBV infection and a specific type of stomach cancer. EBV is found in 8% to 11% of stomach tumors, and EBV-related tumors are more likely than other types of stomach tumors to originate in the gastric cardia (where the contents of the esophagus empty into the stomach), the main body of the stomach, or a gastric stump (the portion of the stomach remaining after partial removal). Statistically significant positive associations between EBV infection (as measured by either antibodies to viral proteins or EBV DNA) and stomach cancer were found in three case-control studies (Shinkura *et al.* 2000, Lo *et al.* 2001, De Aquino *et al.* 2012). Positive associations were also seen in two of three nested case-control studies (Levine *et al.* 1995, Kim *et al.* 2009), but the results were not statistically significant.

Molecular studies provide strong evidence of an association between EBV and some stomach cancers. Monoclonal forms of EBV DNA are found in a subset of human stomach cancers and produce several viral proteins (LMP-1 and -2A and EBNA-1) involved in tumor development. EBV-infected stomach tumors have a unique molecular profile characterized by (1) changes in the DNA chemistry of the tumor-suppressor gene *CDKN2A+* (specifically, addition of methyl groups to control gene expression), resulting in reduced production of two other tumor-suppressor proteins (p16 and 14), (2) mutations in the oncogene *PIK3CA,* and (3) increased numbers of copies of genes involved in cell growth and division (JAK2) and immune suppression (oncogenes *CD274* and *PDCD1LG2*) (Cancer Genome Atlas Research Network 2014, Gulley 2015).

Studies on Mechanisms of Carcinogenesis

Direct evidence that EBV causes lymphoma comes from studies of human cells *in vitro* and of mice *in vivo*. EBV has been shown to transform B lymphocytes into permanently infected lymphoblastoid cell lines in culture (Young and Rickinson 2004) and to transform human epithelial cells co-cultured with cells derived from an EBV-infected Burkitt lymphoma tumor (Imai *et al.* 1998). In addition, EBV-infected B lymphocytes caused B-cell lymphoma in mice with compromised immune systems (Mosier *et al.* 1989, Rowe *et al.* 1991). Normally, newly mature B lymphocytes that are defective are destroyed by undergoing programmed cell death; however, *in vitro* studies have shown that the viral protein EBNA-1 prevents defective B cells from undergoing apoptosis by directly enhancing production of the apoptosis-inhibiting protein survivin (Lu *et al.* 2011) (see also NTP 2016).

Biological Properties

Epstein-Barr virus is an enveloped double-stranded DNA gamma-1 herpesvirus (IARC 2012). The 172-kb EBV genome encodes over 85 genes, categorized as latent or lytic: latent genes are expressed while the virus is dormant, and lytic genes are expressed during the lytic cycle, when the virus replicates and destroys the infected host cell. The two major types of EBV (EBV-1 and EBV-2) differ in the DNA sequences of the genes encoding their nuclear antigens (EBNA-2, -3A, -3B), and each type has several strains. Infection of epithelial cells is primarily lytic, whereas infection of B cells is primarily latent. However, antibody-producing B cells allow EBV to enter the lytic phase and replicate (Ponce *et al.* 2014). EBV proteins produced on the membranes of infected cells in most stages of latency can be recognized by cytotoxic T cells and NK cells, which attack and destroy them (Thorley-Lawson and Gross 2004). However, EBV in latency 0 phase, typically found in resting memory B cells (B lymphocytes sensitized to respond to specific antigens), does not produce proteins on the membranes of infected cells, thereby allowing EBV to evade recognition by the immune system.

Detection

EBV infection can be detected by measuring anti-EBV antibodies in serum or EBV DNA or RNA in peripheral white blood cells (IARC 2012). Measurement of EBV DNA or RNA can indicate EBV viral load, reactivation, response to treatment, and presence in tumor cells. Methods for detection of EBV DNA and RNA include quantitative polymerase chain reaction (PCR), reverse-transcriptase PCR, or *in situ* hybridization. Healthy carriers of EBV do not have detectable EBV DNA or RNA in cell-free serum, so positive results indicate EBV-associated disease or EBV reactivation.

Exposure

Studies measuring antibodies to EBV in blood serum have shown that a significant number of people in the United States are infected with EBV. EBV seroprevalence in the United States, based on National Health and Nutrition Examination Survey data collected in 2009 and 2010, ranged from 50% in 6- to 8-year-olds to 89% in 18- to 19-year-olds (Balfour *et al.* 2013, Dowd *et al.* 2013). Worldwide, more than 90% of adults are infected with EBV (IARC 2012).

Transmission

Transmission of EBV is mainly via saliva, despite the fact that the virus does not infect the salivary glands (IARC 2012). The presence of EBV in peripheral blood suggests that transmission via blood also is possible, and transmission has been reported among transfusion and organ-transplant recipients. Infected cells, primarily resting memory B cells in peripheral blood, provide a permanent reservoir from which progeny viruses can disseminate within the body and infect other people. EBV transmission via breast milk (Daud *et al.* 2015) and genital secretions (Thomas *et al.* 2006) has also been reported.

Age at primary infection varies, occurring at a higher rate during infancy in middle- to low-income countries than in high-income countries, perhaps as a result of better hygienic conditions and other socioeconomic and demographic factors that result in later age of exposure to infected saliva (e.g., household size and population density) (Biggar *et al.* 1978a,b, IARC 2012, Piriou *et al.* 2012, Dowd *et al.* 2013). Similar patterns of lower infection rates (as inferred by seroprevalence) with higher socioeconomic status within race and ethnicity groups are observed in the United States. An analysis of 782 serum samples from Minnesota children aged 18 months to 19.9 years indicated that a combination of genetics, family practices, and home environment were responsible for racial and ethnic differences in EBV prevalence among young children. The route of EBV transmission to preadolescents remains unclear (Condon *et al.* 2014).

Diseases (Non-Cancer), Prevention, and Treatment

Most individuals are infected with EBV but remain otherwise healthy and without symptoms (IARC 2012). Infection is lifelong and has no noticeable symptoms when it occurs in early childhood (IARC 2012); however, it results in infectious mononucleosis in at least 25% of infected teenagers and young adults (CDC 2014b). Oral hairy leukoplakia (a disease of the mucous membranes causing white patches on the tongue) results from infection with EBV in the context of impaired immune function, such as immunosuppression caused by HIV-1 or deterioration of the immune system with normal aging (immunosenescence) (Auwaerter 2015). Chronic active EBV occurs frequently in Asia and South America, but rarely in the United States and Europe. Its etiology is unknown, but is believed to involve rare genetic abnormalities that impair immune control of EBV infection (Rigaud

et al. 2006, Cohen 2009, Chaigne-Delalande *et al.* 2013). EBV has also been suggested as a cause of rare autoimmune diseases affecting the liver, including autoimmune hepatitis (Rigopoulou *et al.* 2012).

EBV transmission is associated with EBV shedding in saliva; therefore, avoiding salivary exposure (e.g., via kissing or sharing food, drink, or toothbrushes) may theoretically prevent transmission (CDC 2014a).

Some drugs have been reported to reduce or inhibit EBV shedding (e.g., Auwaerter 2015); however, no FDA-approved drugs currently exist for treatment of EBV infection. There is no vaccine against EBV, but vaccine development efforts are ongoing (Balfour 2014, ACS 2015, CDC 2015, Cohen 2015, FDA 2015).

Regulations

Department of Transportation (DOT)

Infectious substances are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Food and Drug Administration (FDA)

21 CFR 866 identifies Epstein-Barr virus serological reagents (i.e., devices that consist of antigens and antisera used in serological tests to identify antibodies to EBV in serum) as Class I medical devices requiring premarket notification for FDA clearance to market.

Occupational Safety and Health Administration (OSHA)

Comprehensive regulations have been developed for employers to develop and adhere to exposure control plans for bloodborne pathogens.

All work-related needlestick injuries and cuts from sharp objects that are contaminated with another person's blood or other potentially infectious material must be recorded.

First-aid training program trainees must have adequate instruction in the value of universal precautions for preventing infectious diseases.

Guidelines

Food and Drug Administration (FDA, an HHS agency)

The FDA has issued numerous guidance documents prescribing procedures (e.g., use of standardized labels, abbreviated donor screening questionnaires) for reducing the risk of virus transmission by blood and blood products.

Health Resources and Services Administration (HRSA, an HHS agency)

The Organ Procurement and Transplantation Network (OPTN) prescribes voluntary procedures for donor screening, recordkeeping, notification (e.g., recipient informed consent), and, as appropriate, posttransplant preventive interventions or monitoring and testing procedures to guard against the spread of EBV through solid (vascular) organ transplantation.

American Society of Transplantation (AST)

The AST recommends that EBV serology be performed on all donors and recipients in order to define the risk of posttransplant lymphoma. The AST has issued guidance for the use of solid organs from donors testing positive for EBV and subsequent management of recipients of such organs. No limits are proposed for transplants between donors and recipients who are both EBV positive. AST does advise that EBV-negative recipients of EBV-positive organs be considered for posttransplant nucleic acid test monitoring for EBV to help guide immunosuppression, because they are at a higher risk for primary EBV infection and posttransplant lymphoproliferative disease.

American Red Cross (ARC)

American Red Cross donor eligibility guidelines prohibit blood donation by potential donors who have had hepatitis caused by a virus or unexplained jaundice since age 11, including those who had hepatitis with cytomegalovirus or EBV.

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Hepatitis B Virus

CAS No.: none assigned

Known to be a human carcinogen

First listed in the *Eleventh Report on Carcinogens* (2004)

Also known as HBV

Carcinogenicity

Hepatitis B virus (HBV) is *known to be a human carcinogen* based on sufficient evidence from studies in humans.

Cancer Studies in Humans

Numerous epidemiological cohort and case-control studies conducted in populations differing by race or ethnicity and in various geographic regions have demonstrated that chronic HBV infection causes liver cancer (hepatocellular carcinoma). These studies generally used relatively sensitive and specific serological markers to assess chronic HBV infection. The association between chronic hepatitis B and hepatocellular carcinoma remained strong after adjustment for hepatitis C infection and potential confounding factors such as use of alcohol and tobacco, medical history factors, and dietary exposure to aflatoxin (in areas where aflatoxin contamination of food is common). A meta-analysis of the combined results of 32 studies published between 1993 and 1997 reported a summary odds ratio of 13.7 (95% confidence interval [CI] = 12.2 to 15.4) for development of hepatocellular carcinoma among people chronically infected with HBV (as assessed by the presence of HBV surface antigen in the blood) (Donato *et al.* 1998, NTP 2003).

Cancer Studies in Experimental Animals

HBV infects a limited range of hosts, primarily humans and great apes (Tennant 2001); therefore, studies in experimental animals are limited. In chimpanzees, HBV infection does not appear to increase the risk of liver cancer (Muchmore *et al.* 1990). In studies with transgenic mice carrying HBV genes, liver cancer developed in some, but not all, strains that produced high levels of viral surface antigen or X protein (described below, under Properties), but not in strains expressing the entire HBV genome (NTP 2003).

Studies on Mechanisms of Carcinogenesis

Hepatocellular carcinoma usually emerges after 30 years of chronic HBV infection. During the decades of chronic infection, liver cells undergo many changes as a consequence of ongoing viral replication. Viral DNA becomes integrated into the host cells' DNA through nonhomologous recombination, and the presence of these viral DNA sequences may contribute to development of cancer through multiple steps, by any of several mechanisms. Expression of genes that regulate tissue growth may be altered by DNA sequences located near that gene (*cis*-activation). Viral DNA also may be integrated into the growth-regulatory genes themselves, causing mutation. Integration of viral DNA may result in truncation at the 3′ end of the gene coding for the middle surface antigen or the X protein, resulting in production of novel proteins capable of binding to the *cis*-activating DNA sequences that control gene expression (resulting in *trans*-activation). The majority of HBV-positive hepatocellular carcinomas contain HBV DNA sequences that code for *trans*-activator proteins. Viral integration also may result in general instability of chromosomal DNA. Chromosome allele loss appears to be more frequent in HBV-positive hepatocellular carcinomas than in HBV-negative hepatocellular carcinomas (NTP 2003).

It has also been suggested that some HBV proteins, such as the surface antigens and the X protein, may contribute to tumor formation. In transgenic mice, overproduction of the HBV large surface antigen leads to persistent inflammation, production of oxygen radicals, and DNA damage. The X protein may activate viral and host-cell promoters and signal transduction pathways, inhibit DNA repair, and affect the cell cycle and apoptosis. High levels of the X protein may transform immortalized mouse fibroblast (3T3) cells (NTP 2003).

Biological Properties

HBV is an enveloped DNA virus that infects hepatocytes, causing hepatitis B (Blum *et al.* 1983). It is a member of the Hepadnaviridae family, which includes the genera *Orthohepadnavirus* (infecting mammals) and *Avihepadnavirus* (infecting birds). The hepadnaviruses have a characteristic partially double-stranded DNA genome, which is held in a circular conformation by a short, cohesive overlap between the 5′ ends of the two strands (Ganem and Schneider 2001). The HBV genome codes for seven proteins: viral DNA polymerase, the core protein (hepatitis B core antigen, HBcAg), the precore protein, the X protein, and three viral envelope (surface antigen, HBsAg) proteins, large (L), middle (M), and small (S) (NTP 2003). The virion has an icosahedral nucleocapsid, composed of core protein enclosing the viral genome. The nucleocapsid is surrounded by an envelope 42 nm in diameter, which contains (in order of decreasing abundance) the L, M, and S proteins. L is thought to specify the virus's host range, by recognizing cell surface receptors, and S is the immunodominant component of the envelope. The functions of the precore and X proteins are unknown. However, it has been proposed that X affects a variety of cell processes, which may in turn significantly affect hepatocyte gene expression, cell survival, and viral replication. The precore protein is cleaved to form a soluble protein (hepatitis B envelope antigen), which is secreted from infected cells and may be detected in the blood of infected individuals (Seeger and Mason 2000, Ganem and Schneider 2001).

Detection

HBV infection is confirmed by detection of HBV proteins, antibodies against HBV proteins, or HBV DNA in the blood. The detection of different proteins and antibodies against these proteins are indicators of different stages of infection. The presence of HBsAg indicates acute or chronic HBV infection, whereas the presence of anti-HBsAg antibodies indicates immunity (due to resolved infection or vaccination) (Hollinger and Dienstag 1995). Strictly speaking, chronic HBV infection is defined by detection of serum HBsAg in two tests six months apart; however, this criterion is not practical for most epidemiological studies. Because adults who are not carriers of HBV are highly unlikely to test positive for HBsAg, a single positive test result is considered a valid indicator of chronic carrier state in epidemiological studies. Assays approved by the U.S. Food and Drug Administration include enzyme or chemiluminescent immunoassays for HBsAg and anti-HBcAg antibodies and polymerase chain reaction assays for HBV nucleic acid (FDA 2016).

Exposure

Studies measuring antibodies to HBV in blood serum have shown that a significant number of people in the United States are chronically infected with HBV. At least 565,000 and up to 1,130,000 people living in the United States (not including those residing in institutions) have a chronic HBV infection (National Health and Nutrition Examination Survey, as reported by Roberts *et al.* 2016). Since 1999, the overall prevalence of chronic HBV infection has remained constant at 0.3% (95% CI = 0.2% to 0.4%). Chronic HBV infection is about twice as prevalent among non-Hispanic blacks (0.6%, 95% CI = 0.4% to 1.0%) and about 10 times as prevalent among non-Hispanic Asians (3.1%, 95% CI = 1.8% to 5.2%) as among the general public. The Centers for Disease Control and Prevention estimates that over 19,200 people had an acute HBV infection in 2014; however, the number of new cases of acute infection per year has decreased from 13.8 per 100,000 in 1987 to 1.0 per 100,000 in 2013 (Goldstein *et al.* 2002, CDC 2015, 2016). Screening of the U.S. blood supply began with a test for HBsAg in 1971, followed by a test for hepatitis B core antibody in 1986, and most recently nucleic acid testing for HBV in 2009.

Worldwide, over 240 million people have chronic hepatitis B, and more than 686,000 people die each year from HBV-related disease (including cirrhosis and liver cancer) (WHO 2016). The prevalence of chronic infection varies geographically, ranging from low (less than 1%) in Western Europe and North America, to intermediate (2% to 5%) in the Middle East and the Indian subcontinent, to highest (5% to 10%) in sub-Saharan Africa and East Asia, with high rates also reported for the Amazon and the southern parts of eastern and central Europe.

Transmission

The major routes of HBV transmission are parenteral (primarily by injection or transfusion), through sexual contact, from mother to infant at the time of birth, and through health-care practices (Hollinger and Liang 2001). Mother-to-infant transmission is important primarily in areas where HBV infection is endemic. In U.S. surveillance studies conducted in 1992–93, most cases resulted from heterosexual transmission (41%), followed by intravenous drug use (15%) and homosexual transmission (9%); however, 31% of HBV infections were not associated with any known risk factors (CDC 2002). Because the U.S. blood supply is screened for HBV, the estimated risk of infection via blood transfusion is about one in 800,000 to one in a million per transfused unit (American Red Cross 2016). A risk also exists for transmission of HBV from HBV-infected donors to unininfected recipients of solid (vascular) organ transplants (HHS 2013). The American Society of Transplantation guidelines state that decisions to transplant organs from HBV-infected patients to uninfected adults should be made on a case-by-case basis that considers the risks and benefits, and with use of antiviral prophylaxis to reduce the rate of transmission of HBV to liver recipients (Huprikar *et al.* 2015).

Diseases (Non-Cancer), Prevention, and Treatment

HBV infection can cause acute or chronic hepatitis B. Acute hepatitis B is characterized by tissue changes, including hyperplasia, inflammation, and cell death, which appear to result from the host's immune response to HBV antigen (IARC 1994). Chronic hepatitis B, defined as the presence of circulating HBsAg for over six months, develops in individuals with acute hepatitis B who are not able to clear the virus. The risk of chronic hepatitis B among HBV-infected individuals appears to depend on the status of the immune system at the time of infection and is much higher in HBV-infected infants and children than in HBV-infected adults. About 70% to 90% of infants infected before one year of age develop chronic hepatitis B, whereas the risk of chronic infection among HBV-infected adults is 5% to 10% (IARC 1994, Hollinger and Liang 2001). In chronic hepatitis B, the patient's immune response to HBV results in cycles of cell death and regeneration that may progress to fibrosis of the liver and cirrhosis (replacement of normal liver tissue with bands of fibrous tissue surrounding nodules of regenerating liver tissue) (Hollinger and Liang 2001).

HBV infection can be prevented by screening of the blood supply, reduction of contact with potentially contaminated fluids in health-care settings, and vaccination. The Occupational Safety and Health

Administration has established a bloodborne pathogens standard, based on the concept of universal precautions, which requires that body fluids and materials be treated as infectious (OSHA 1992). Recombinant hepatitis B vaccines, which contain HBsAg (produced by genetically engineered yeast cells), have been available in the United States since the 1980s and are recommended for all infants and for individuals at high risk (Hollinger and Liang 2001). From 1999 to 2012, the overall adjusted prevalence of vaccine-induced immunity for hepatitis B virus increased from 21.7% (95% CI = 20.8% to 22.7%) in 1999 to 2006 to 25.1% (95% CI = 24.1% to 26.1%) in 2007 to 2012. Hepatitis B is treated with immunomodulators (drugs that affect the immune system and are not specific for HBV), antiviral drugs, and combination therapy with both drug types; however, these drugs have limited efficacy (Hollinger and Liang 2001, Schalm *et al.* 2002).

Regulations

Department of Transportation (DOT)

Infectious substances are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Food and Drug Administration (FDA, an HHS agency)

Regulations have been established to guard against the spread of hepatitis B virus through donation of blood, serum, and human immune globulin, including requirements for donor screening, product testing, and product labeling.

Each donation of blood, plasma, or serum to be used in preparing a biological product shall be tested for the presence of hepatitis B surface antigen.

21 CFR 1270 and 1271 prescribe procedures, including donor screening and tissue testing, to ensure that tissues intended for human transplant or other human cells, tissues, and cellular and tissue-based products are free of hepatitis B virus.

Occupational Safety and Health Administration (OSHA)

Comprehensive regulations have been developed for employers to develop and adhere to exposure control plans for bloodborne pathogens.

An employer shall make the hepatitis B vaccine available to employees who have had exposure to pathogenic microorganisms.

All work-related needlestick injuries and cuts from sharp objects that are contaminated with another person's blood or other potentially infectious material must be recorded.

First-aid training program trainees must have adequate instruction in the value of universal precautions for preventing infectious diseases.

Public Health Service (PHS, a division of HHS)

Regulations have been established to control the spread of hepatitis from hemodialysis treatment.

Guidelines

American Society of Transplantation (AST)

The AST has issued guidance for the use of solid organs from donors testing positive for HBV and subsequent management of recipients of such organs.

Department of Health and Human Services (DHHS)

DHHS has issued an updated Public Health Service (PHS) guideline that prescribes donor screening and notification (e.g., recipient informed consent) procedures to guard against transmission of HBV through organ transplants. This guidance also includes a revised set of risk factors for HBV infection to help improve recipient informed consent and prompt more sensitive laboratory testing of donors and recipients when necessary.

Health Resources and Services Administration (HRSA, an HHS agency)

The Organ Procurement and Transplantation Network (OPTN) prescribes donor screening, recordkeeping, notification, and organ and vessel packaging, labeling, shipping, and storage procedures to guard against the spread of hepatitis B virus through solid (vascular) organ transplantation.

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Hepatitis C Virus

CAS No.: none assigned

Known to be a human carcinogen

First listed in the *Eleventh Report on Carcinogens* (2004)

Also known as HCV

Carcinogenicity

Hepatitis C virus (HCV) is *known to be a human carcinogen* based on sufficient evidence from studies in humans.

Cancer Studies in Humans

In epidemiological research, numerous cohort and case-control studies conducted in populations differing by race or ethnicity and in various geographical locations have demonstrated that chronic HCV infection causes liver cancer (hepatocellular carcinoma) (NTP 2003). A meta-analysis of 32 studies published between 1993 and 1997 reported a summary odds ratio of 11.5 (95% confidence interval = 9.9 to 13.3) (Donato *et al.* 1998), meaning that patients with chronic HCV infection were 11.5 times as likely as uninfected individuals to develop hepatocellular carcinoma. These studies generally used

relatively sensitive and specific serological markers (anti-HCV antibodies or HCV RNA in the blood) to assess chronic HCV infection. The association between HCV and hepatocellular carcinoma was independent of hepatitis B virus (HBV) infection, and it remained when studies controlled for potential confounders such as the use of alcohol or tobacco. A number of recent studies have investigated whether some genotypes of HCV may be more potent carcinogens than others. Although the results are not entirely consistent, the evidence generally supports the hypothesis that HCV genotype 1b is more strongly associated with hepatocellular carcinoma than are other HCV genotypes. A number of recent case-control studies and one cohort study have linked HCV infection to increased risk of B-cell lymphoma; however, many of these studies had relatively small sample sizes, and all were hospital-based (NTP 2003). In 1994, the International Agency for Research on Cancer classified HCV as carcinogenic to humans based on sufficient evidence of carcinogenicity in humans (IARC 1994).

Cancer Studies in Experimental Animals

Studies of HCV in experimental animals are limited, because the only animals known to be susceptible to HCV infection are chimpanzees and tree shrews. Liver cancer (hepatocellular carcinoma) was reported in one chimpanzee that had been infected with HCV for seven years, but not in HCV-infected tree shrews (Linke *et al.* 1987, Muchmore *et al.* 1988, Xie *et al.* 1998). Hepatocellular carcinoma also developed in a few lines of transgenic mice carrying HCV genes; the cancer was observed primarily in males producing either the HCV core protein or low levels of the complete HCV polyprotein (components of the HCV virus, as discussed under Properties, below) (Moriya *et al.* 1998, Koike *et al.* 2002, Lerat *et al.* 2002).

Studies on Mechanisms of Carcinogenesis

The mechanism(s) by which HCV causes liver cancer has not been determined. HCV may cause cancer directly or indirectly, the latter as a result of liver inflammation and regeneration associated with chronic hepatitis. As an RNA virus, HCV does not integrate into the DNA of the hepatitis patient's cells; therefore, direct mechanisms of carcinogenesis would most likely involve the effects of viral protein on cell growth (Fong *et al.* 1991). The HCV core protein is the current leading suspect, based on its role in regulating cellular promoters of gene expression and proto-oncogenes and on the studies in transgenic mice mentioned above. Studies with cell cultures have shown that the HCV core protein cooperates with the *ras* oncogene to transform primary rat embryo fibroblasts to a tumorigenic phenotype (Ray *et al.* 1996). The roles of other HCV proteins in causing liver cancer remain largely unexplored. HCV-related liver cancer almost always arises in the presence of cirrhosis of the liver, suggesting the importance of indirect mechanisms such as inflammation, fibrosis, and hepatocyte regeneration in the development of cancer (Craig *et al.* 1991, Bralet *et al.* 2000). It is hypothesized that cirrhosis results in hepatocellular carcinoma when nodules within the cirrhotic liver become dysplastic (i.e., precancerous cells develop within the nodules) (Takayama *et al.* 1990). Several studies (though not all) have reported an association between HCV-associated liver cancer and β-catenin gene mutations (which are associated with other types of cancer); however, these studies were based on small numbers of tumors (Huang *et al.* 1999, Laurent-Puig *et al.* 2001, Ueta *et al.* 2002).

Biological Properties

HCV is an enveloped RNA virus, which causes most non-B viral hepatitis that is transmitted parenterally (i.e., by injection, transfusion, or other contact with body fluids). It is a member of the Flaviviridae family of viruses and has a particle size of about 50 nm in diameter (He *et al.* 1987). The positive-sense RNA genome (9,600 nucleotides) codes for production of a polyprotein (3,000 amino acids); enzymes produced by the virus and the host cell then cleave the polyprotein into the smaller structural and nonstructural proteins that make up the mature virus particle. The structural proteins, which are incorporated into the viral envelope, consist of the core (nucleocapsid) protein and two glycoproteins (E1 and E2). The nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) serve as enzymes essential for protein processing and RNA replication; their functions include protease, nucleotide triphosphatase, RNA helicase, and RNA polymerase activity (Rosenberg 2001).

Replication of HCV often results in random mutations that are not corrected by the RNA polymerase because it lacks a proofreading function. As a result, the genomes of HCV strains show extensive variability. However, some regions of the genome are more variable than others, and classification of HCV genotypes is based on differences in the less variable regions of the genome. HCVs can be divided into six phylogenetically distinct groups designated as clades (groups of genotypes that share a common ancestor). Within the clades, a number of subtypes (individual genotypes) have been defined (Simmonds *et al.* 1993, Bukh *et al.* 1995, Simmonds 1995, Robertson *et al.* 1998). All known types of HCV have the potential to cause serious liver disease.

Detection

HCV infection usually is confirmed by detection of antibodies against HCV proteins or by detection of HCV RNA. Anti-HCV antibodies are detected by serological assays, and HCV RNA usually is detected by tests based on the polymerase chain reaction.

Exposure

Studies measuring antibodies to HCV in serum have shown that a significant number of people in the United States are infected with HCV. Between 2.2 million to 3.2 million people (0.8% to 1.2% of people living in the United States who were not residing in institutions) were infected with HCV during the years 2003 to 2010 (National Health and Nutrition Examination Survey, as reported by Denniston *et al.* 2014). These data showed economic disparity in chronic HCV infection; it was most common among non-Hispanic black men aged 40 to 49 years who were not educated beyond high school and whose family income was below twice the poverty level (Denniston *et al.* 2014). The most common risk factors for HCV infection were illicit drug use (including injection drug use) and a history of blood transfusion before 1992; however, 49% of HCV-infected individuals did not report either risk factor.

The worldwide prevalence of HCV seropositivity has been estimated to be at least 92 million (1.6%) (Gower *et al.* 2014) and may be as high as 170 million (3%) (Holtzman 2015). Prevalence varies geographically, ranging from 0.2% to 2.0% in Western Europe and most other developed countries in Asia and Latin America, but higher rates have been reported for individual countries in central Asia, Eastern Europe, and sub-Saharan Africa (Gower *et al.* 2014). Prevalence was highest in Egypt (14.7%), but between 0.5% and 3.2% in other North African or Middle Eastern countries.

Transmission

The major route of HCV transmission is through contaminated blood. Because the U.S. blood supply is screened for HCV, the estimated risk of infection via blood transfusion is about one in a million per transfused unit (American Red Cross 2016). Screening of the U.S. blood supply began with a test for HCV antibody in 1990, and nucleic acid testing

for HCV began in 1999. The major risk factor for infection is illegal intravenous drug use. Other routes of transmission include sexual, perinatal, familial (at low rates), and through health-care practices, including transmission by contaminated equipment or supplies, from patient to patient (at low rates), and through occupational exposure (at low rates). In U.S. surveillance studies from 1983 to 1996, no epidemiological risk factors were identified for at least 10% of the cases of acute hepatitis C (Alter *et al.* 1999, Major *et al.* 2001). The American Society of Transplantation (AST) strongly discourages transplantation of an HCV-positive organ into an HCV-negative recipient, because of extremely poor outcomes and the near certainty of transmission of HCV. The AST also advises stringent informed consent guidelines with any use of an HCV-positive donor (e.g., for a critically ill patient awaiting a life-sustaining transplant, or transplantation of an HCV-positive organ into an HCV-positive recipient) (Fischer *et al.* 2013, Levitsky *et al.* 2013).

Diseases (Non-Cancer), Prevention, and Treatment

HCV can cause acute or chronic hepatitis. Acute hepatitis C usually is characterized by elevated or fluctuating levels of alanine transaminase (ALT). People with acute hepatitis C either have no symptoms (60% to 70%) or have mild clinical disease symptoms: 10% to 20% have nonspecific symptoms, such as nausea, vomiting, anorexia, or abdominal pain, and 20% to 30% may become jaundiced. The average time from exposure to symptoms is six to seven weeks (MMWR 1998). Most people infected with HCV (75% to 80%) go on to develop chronic hepatitis C. Individuals with chronic hepatitis C are the source for all new infections and are at increased risk for chronic liver disease, cirrhosis, and liver cancer (Bonkovsky and Mehta 2001). Chronic hepatitis is associated with chronic liver injury and inflammation. Liver injury appears to be a result of the patient's immune reaction to the virus, rather than damage by the virus itself. Chronic infection usually results in progressive fibrosis of the liver, which may progress to cirrhosis and other disease states. In the United States, HCV is the leading cause of liver disease and may account for 8,000 to 10,000 deaths per year. As of 1996, most HCV-infected individuals were between 30 and 49 years of age; thus, the number of deaths could substantially increase during the next 20 to 30 years, as this group reaches the age at which complications from liver disease usually occur (MMWR 1998, Alter *et al.* 1999). The World Health Organization has estimated that 700,000 people die each year from hepatitis C–related liver diseases (WHO 2016); the most common causes of death are cirrhosis and liver cancer (Denniston *et al.* 2014).

HCV infection can be prevented by screening of the blood supply and reduction of contact with potentially contaminated fluids in health-care settings. The Occupational Safety and Health Administration has established a bloodborne pathogens standard, based on the concept of universal precautions, which requires that body fluids and materials be treated as infectious (OSHA 1992). Currently, HCV is treated with interferon-based therapies, and no vaccine is available.

Regulations

Department of Transportation (DOT)

Infectious substances are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Food and Drug Administration (FDA, an HHS agency)

Regulations have been established to guard against the spread of hepatitis C through donation of blood, serum, and human immune globulin, including requirements for donor screening, product testing, and product labeling.

Regulations in 21 CFR 1270 and 1271 prescribe procedures, including donor screening and tissue testing, to ensure that tissues intended for human transplant or other human cells, tissues, and cellular and tissue-based products are free of hepatitis C.

Each donation of blood or blood product to be used in preparing a biological product shall be tested for the presence of hepatitis C surface antigen.

Occupational Safety and Health Administration (OSHA)

All work-related needlestick injuries and cuts from sharp objects that are contaminated with another person's blood or other potentially infectious material must be recorded.

First-aid training program trainees must have adequate instruction in the value of universal precautions for preventing infectious diseases.

Comprehensive regulations have been developed for employers to develop, and adhere to, exposure-control plans for bloodborne pathogens.

Public Health Service (PHS, a division of HHS)

Regulations have been established to control the spread of hepatitis from hemodialysis treatment.

Guidelines

American Society of Transplantation (AST)

The AST has issued guidance to guard against the spread of HCV through solid (vascular) organ transplantation.

Department of Health and Human Services (DHHS)

DHHS has issued an updated Public Health Service (PHS) guideline that prescribes donor screening and notification (e.g., recipient informed consent) procedures to guard against transmission of HCV through organ transplants. This guidance also includes a revised set of risk factors for HCV infection to help improve recipient informed consent and prompt more sensitive laboratory testing of donors and recipients when necessary.

Health Resources and Services Administration (HRSA, an HHS agency)

The Organ Procurement and Transplantation Network (OPTN) prescribes donor screening, recordkeeping, notification, and organ and vessel packaging, labeling, shipping, and storage procedures to guard against the spread of HCV through solid (vascular) organ transplantation.

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Human Immunodeficiency Virus Type 1

CAS No.: none assigned

Known to be a human carcinogen

Also known as HIV-1

Carcinogenicity

Human immunodeficiency virus type 1 (HIV-1) is known to be a human carcinogen based on sufficient evidence from studies in humans. This conclusion is based on epidemiological studies showing that HIV-1 increases the risk of Kaposi sarcoma, non-Hodgkin lymphoma, Hodgkin lymphoma, cervical cancer, invasive anal cancer, genital cancers (vaginal/vulvar and penile cancers), conjunctival eye cancer, and non-melanoma skin cancer, together with supporting evidence from mechanistic studies demonstrating the biological plausibility of its carcinogenicity in humans. Epidemiological studies also provide limited evidence for an association between HIV-1 infection and oral-related (oral cavity and oropharyngeal), lung, and liver cancers.

The majority of these 12 types of cancer are considered to be related to co-infection with both HIV-1 and another cancer-causing virus. HIV-1 infection impairs the body's immune system so that it cannot adequately suppress or destroy cancer-causing viruses, resulting in an increased risk that these viruses will cause cancer in co-infected individuals. Discussion of the types of cancer associated with HIV-1 infection (below) is organized by whether or not they are related to co-infection with another virus (CDC 1992, Gopal *et al.* 2014, Patel *et al.* 2014). Three of the nine infection-related cancers (Kaposi sarcoma, non-Hodgkin lymphoma, and cervical carcinoma) are types of cancer whose presence has been used to diagnose acquired immunodeficiency syndrome (AIDS) and are known as AIDS-defining cancers. AIDS is a disease caused by HIV-1 that attacks the body's immune system by reducing the number of CD4 T helper cells, which help the body fight off infection. Viral co-infections have not been identified for lung cancer, conjunctival eye cancer, or non-melanoma skin cancer; however, viral co-infection is likely involved in one type of non-melanoma skin cancer (Merkel cell carcinoma).

The impact on public health of HIV-1-related cancers is a major concern, as the excess number of cancer cases due to HIV-1 infection in the United States in 2010 was estimated to be over 3,900 (Robbins *et al.* 2015). In recent years, several studies have reported that HIV-1-infected individuals have increased risks for non-AIDS-defining cancers as a group, as well as for additional specific types of cancer, especially those not thought to be related to co-infection with other viruses (Shiels *et al.* 2009, Albini *et al.* 2013, Franzetti *et al.* 2013, Helleberg *et al.* 2015). Nonetheless, about 90% of the excess cancer due to HIV-1 infection identified by Robbins *et al.* is accounted for by the types of cancer described in this profile.

Infection-Related Cancers

Cancer Studies in Humans

Evidence for associations between HIV-1 infection and Kaposi sarcoma, non-Hodgkin lymphoma, Hodgkin lymphoma, cervical cancer, invasive anal cancer, vaginal/vulvar cancer, and penile cancer is based on consistent findings of statistically significant increased risk in numerous epidemiological studies in different populations. At least 30 cohort studies published through 2015 with relatively large numbers of HIV-1/AIDS cases (NTP 2016) found that people with AIDS or infected with HIV-1 had moderate to very high increased risks for most of these types of cancer, compared with the general population or with HIV-1-negative individuals. Although the general population may differ from HIV-1 infected individuals with respect to lifestyle-related risk factors for specific types of cancer (i.e., potential confounding factors), the overwhelming strength of the associations between these cancers and HIV-1 infection in numerous studies eliminates concern that the increased risks are explained by these potential confounding factors.

The evidence for vaginal/vulvar and penile cancers, which are rare, is based on a smaller number of studies (six or seven) for each type of cancer (Newnham *et al.* 2005, Mbulaiteye *et al.* 2006, Long *et al.* 2008, Patel *et al.* 2008, Chaturvedi *et al.* 2009, Dal Maso *et al.* 2009, Simard *et al.* 2010, Franzetti *et al.* 2013, Park *et al.* 2014, Raffetti *et al.* 2015). In general, similar risk estimates for penile cancer were found across different HIV-1 risk groups (injection drug users, heterosexuals, and men having sex with men), which helps to rule out potential confounding by lifestyle behaviors as the cause of the excess cancer risk observed in these studies (Chaturvedi *et al.* 2009).

For each type of infection-related cancer, the table on the next page summarizes the level of evidence, the risk estimates from the studies, the cancer-causing virus with which the patients were infected, and whether the risk increased with low CD4 cell counts (which indicate impaired immune function) (NTP 2016).

There is limited evidence from studies in humans for a causal association between HIV-1 infection and oral-related cancer (oral-cavity

Type of cancer	Level of evidence	Risk estimates	Cancer-causing viral co-infection	Increased risk with low CD4 cell counts[a]?
Kaposi sarcoma	sufficient/AIDS-defining	100s to 10,000s	KSHV	Yes
Non-Hodgkin lymphoma	sufficient/AIDS-defining	10 to ~300	EBV, KSHV[b]	Yes
Hodgkin lymphoma	sufficient	4 to 38	EBV	Yes
Cervical	sufficient/AIDS-defining	2 to 22	HPV	Unclear; CIN = yes
Anal	sufficient	10 to 100	HPV	Yes
Vaginal/vulvar	sufficient	5 to 27	HPV	Yes
Penile	sufficient	4 to 28	HPV	Unclear
Oral-related	limited	2 to 4	HPV	Unclear
Liver	limited	2 to 16	HCV/HBV	Yes

CIN = cervical intraepithelial neoplasia; EBV = Epstein-Barr virus; HBV = hepatitis B virus; HCV = hepatitis C virus; HPV = human papillomavirus; KSHV = Kaposi sarcoma-associated herpesvirus.
[a]Low CD4 counts are a measure of an impaired immune system.
[b]Some types of non-Hodgkin lymphoma, primary effusion lymphoma, and the plasmablastic variant of multicentric Castleman disease.

and oropharyngeal cancers). At least 19 cohort studies found that HIV-1-infected people had 2- to 4-fold higher risks for oral-related cancer (all types combined, oropharyngeal cancer, or specific oral-cavity cancers) than did the general population or HIV-1-negative individuals; two studies reported risks over 10-fold higher for cancers of the tonsil or tongue (NTP 2016). Interpretation of these modestly increased risks is complicated by the fact that different subtypes of oral-related cancers, which were combined in most HIV studies, may develop by different mechanisms. The link between oral cancer and human papillomavirus (HPV) is likely to depend on the specific type of oral cancer. Risk factors (such as sexual activity) for HPV-related oropharyngeal cancer are thought to differ from those for non-HPV-related oral-cavity cancers (such as cigarette smoking and alcohol consumption) (Gillison *et al.* 2008). HPV-related cancers are more prevalent among HIV-1-infected than HIV-1-negative individuals, either because they differ with respect to risk factors for HPV or, possibly, because of the immunosuppressive effects of HIV-1 (Beachler and D'Souza 2013). In addition, most studies did not measure or control for other risk factors for oral cancer. In the only study identified that evaluated tobacco smoking, HIV-1 infection, and oropharyngeal cancer in HIV-1-positive and -negative individuals, controlling for smoking reduced the risk estimate from 1.9 to 1.4 (Silverberg *et al.* 2011).

Epidemiological studies also provide limited evidence for an association between HIV-1 infection and liver cancer (primarily hepatocellular carcinoma). At least 40 studies reported an increased risk (2- to 16-fold) of liver cancer among HIV-1-positive individuals; however, it is not possible to reasonably rule out the possibility that the excess risk can be explained by biases in these studies (NTP 2016). In the United States, hepatitis B virus (HBV) and hepatitis C virus (HCV) are more common among HIV-1-infected individuals than in the general population. Therefore, it is not clear whether HIV-1 infection is a potential confounding factor, because it is correlated with HBV or HCV infection, or whether it plays an active role in the development of HBV- and HCV-related liver cancer, primarily by suppressing the immune system. There is evidence to suggest that progression to end-stage liver disease or liver cancer is more aggressive in individuals co-infected with HIV-1 and HBV or HCV (Mohsen *et al*. 2002, Bourcier *et al.* 2012). In addition, several studies have found the risk of liver cancer to be associated with immune-system suppression, as measured by low CD4 cell counts, which would support a role for HIV-1 in cancer development (Engels *et al.* 2008, Guiguet *et al*. 2009, Silverberg *et al.* 2011, Vogel *et al.* 2011, Kramer *et al.* 2015). Some studies investigating the risk of liver cancer among individuals co-infected with HIV-1 and HCV have found no increased risk associated with HIV-1 co-infection or after controlling for HCV, arguing against a causal role for HIV-1 (Kramer *et al.* 2005, McGinnis *et al.* 2006, Di Benedetto *et al.* 2014). However, a limitation of these studies is that the cohorts may not have been followed up long enough for them to have developed liver cancer, which has a long period between initial exposure and development of cancer.

Studies on Mechanisms of Carcinogenesis

The available data support a mechanism of carcinogenesis in which an HIV-1-impaired immune system cannot adequately suppress or destroy cancer-causing viruses, resulting in increased risks of cancers caused by these viruses (NTP 2016). The risks of most (though not all) of the 12 types of cancer discussed above are related to decreased CD4 cell count; however, the relationships for the different types of cancer may depend on the timing of the decrease in CD4 cell count. In addition, high mortality from some types of cancer (such as cervical) during the early years of the AIDS epidemic, before the introduction of highly active antiretroviral therapy (HAART, more recently referred to as combined antiretroviral therapy, cART), might have masked the relationship between cancer risk and CD4 cell count (Chaturvedi *et al.* 2009). Abnormalities in cervical cells and an increased cancer risk from precancerous cervical lesions have been associated with low CD4 counts in several studies (Denslow *et al.* 2014).

HAART, which reduces the level of HIV-1 in the blood, has substantially decreased the risks of Kaposi sarcoma and non-Hodgkin lymphoma, supporting the link between HIV-1 infection and increased risk of these cancers. However, the cancer risks remain higher among HIV-1-infected individuals than among non-HIV-1-infected individuals (Shiels *et al.* 2011a,b). In contrast, the risks of Hodgkin lymphoma, invasive anal cancer, and possibly the genital cancers have increased with the advent of HAART, in part because people with HIV-1 now survive longer. This results in a larger and older population of HIV-1-positive individuals, thus increasing the chance that these types of cancer will develop and be observed. In addition, the toxicity of some of the antiretroviral drugs used in HIV-1/AIDS treatment may increase the risk of cancer (Borges *et al.* 2014). The effect of HAART is less clear for cervical cancer; some, but not all, studies have found decreased risk since the advent of HAART.

Impaired immune function alone clearly does not fully explain the incidences of cancer and range of cancer types observed among HIV-1-infected individuals either before or after the advent of HAART. Although HAART improves immune function and lowers the level of HIV-1 in the blood, it only partially reduces the inflammation associated with HIV-1 infection, suggesting that inflammation and other molecular pathways may contribute to the increased cancer risk (Borges *et al.* 2013, 2014). Some studies showed that cumulative or current levels of HIV-1 RNA in the blood were associ-

ated with an increased risk of AIDS-defining cancers independently of other risk factors, or that specific HIV-1 proteins (e.g., Tat and Vpr) might work synergistically with other cancer-causing viruses (Borges *et al.* 2014).

Cancers Not Known To Be Infection-Related

Cancer Studies in Humans

Evidence for associations between HIV-1 infection and conjunctival eye cancer and non-melanoma skin cancer is based on consistent findings of statistically significant increased risks in numerous epidemiological studies in different populations. The increased risks have ranged from moderate to high. The evidence for lung cancer is limited, because potential confounding from smoking could not be completely ruled out as the cause of lung cancer in these studies.

The evidence for an association of HIV-1 infection with conjunctival eye cancer comes from at least four cohort studies and four case-control studies (IARC 2012, NTP 2016) that reported positive associations, with relative risks ranging from 12 to 15 in most of the studies.

Over 15 studies have reported increased risks of non-melanoma skin cancer associated with HIV-1 infection, ranging mostly from 1.5- to 6-fold, but up to 20-fold in a few studies (NTP 2016). A meta-analysis that combined the findings of six cohort studies of people with HIV-1/AIDS published between 2003 and 2013 reported an overall relative risk of 2.76 (95% confidence interval = 2.55 to 2.98) (Zhao *et al.* 2015). In addition, a cohort study found a positive association between the level of HIV-1 RNA in the blood and the risk of non-melanoma skin cancer, suggesting an exposure-response relationship (Crum-Cianflone *et al.* 2015). Increased incidences of Merkel cell carcinoma, a rare form of non-melanoma skin cancer caused by Merkel cell polyomavirus (MCV), have been found in HIV-1-infected individuals in some studies (Engels *et al.* 2002), suggesting that this type of non-melanoma skin cancer can be considered an infection-related cancer. However, no studies have measured both MCV and HIV-1 viruses in Merkel cell carcinoma patients, so it is not known whether Merkel cell carcinoma is more likely to occur in people co-infected with both viruses than in those infected with a single virus.

The majority of studies (at least 48) have reported a positive association between HIV-1 infection and lung cancer (NTP 2016). Most of these studies reported statistically significant increased risks of approximately 1.5- to 6-fold. It has been suggested that tobacco smoking could explain the excess risk, because many of these studies compared lung cancer incidence between HIV-1-infected cohorts (with 40% to 80% smokers) and the general population, where the prevalence of smoking is typically much lower (20% to 40%). However, almost all studies that controlled for smoking (Phelps *et al.* 2001, Engels *et al.* 2006, Kirk *et al.* 2007, Shiels *et al.* 2010, Sigel *et al.* 2012, Hessol *et al.* 2015) or used statistical models to estimate the effect of smoking on cancer risk (Charturvedi *et al.* 2007) found the risks of lung cancer incidence or death to be at least doubled, and most of the increased risks were statistically significant. Although one study did not find an increased risk of lung cancer among the total HIV-1-infected population after adjusting for smoking (Silverberg *et al.* 2011), increased risks were found among those HIV-1-infected individuals whose blood had the highest levels of HIV-1 RNA (> 10,000 copies/mL) or lowest CD4 cell counts (≤ 200 cells/μL, the cutoff CD4 count for an AIDS diagnosis). The evidence suggests that tobacco smoking does not explain all of the excess risk of lung cancer among people infected with HIV-1. However, because smoking may not have been measured precisely in these studies, the possibility that smoking was the sole cause of lung cancer in these HIV-1-infected individuals could not reasonably be ruled out.

Studies on Mechanisms of Carcinogenesis

Mechanisms of carcinogenicity for these non-infection-related, non-AIDS-defining cancers are unclear, but (as with the AIDS-defining cancers) may be related to impaired immune function and inflammation in people infected with HIV-1 (NTP 2016). In addition, traditional risk factors (such as smoking, alcohol abuse, exposure to ultraviolet radiation, and age) may play a primary role in or contribute to the increased risk of non-AIDS-defining cancers in people with HIV-1 (Silverberg and Abrams 2007, Engels 2009, Shiels *et al.* 2011a, Borges *et al.* 2014). Furthermore, evidence is emerging for a direct carcinogenic effect of HIV-1 and some of its proteins, such as disruption of the cell-division cycle, inhibition of tumor-suppressor genes, promotion of chromosome instability, inhibition of DNA repair, and promotion of the carcinogenic effects of other agents (Borges *et al.* 2014).

Biological Properties

HIV-1 was first identified as the virus associated with AIDS in 1983. It is an enveloped single-stranded RNA retrovirus of the subfamily Orthoretrovirinae and genus *Lentivirus* (IARC 1996, 2012). HIV-1 is composed of an outer lipid membrane envelope with two surface proteins surrounding a protein matrix, inside of which is a protein capsid (shell) containing two copies of the 9.8-kb viral genome and the enzymes for viral replication, integration into host-cell genetic material, and processing of viral proteins.

HIV-1 infections are typically characterized by a long delay before the emergence of symptoms (IARC 1996, 2012, DHHS 2015). HIV-1 infects mainly CD4 cells, and also other cells of the immune system, including B cells, monocytes, macrophages, and follicular dendritic cells (IARC 1996, 2012). The immune system responds with increased production of CD8 (killer) T cells and antibodies that kill infected CD4 cells and other infected white blood cells. CD4 cells are also killed by viral replication and disruption of cell regulation. After an initial peak of infection, the amount of HIV-1 in the blood decreases (IARC 1996, 2012, CDC 2016a). The virus can then remain at low levels for 2 to 25 years, averaging about a decade, and can evade detection by the immune system through several mechanisms, including producing proteins that prevent the immune system from detecting the virus.

Detection

HIV-1 has been detected primarily in blood and sexual fluids (semen and vaginal secretions) and in very low concentrations in other body fluids, including saliva, urine, sweat, and tears (which may show higher concentrations if they have been contaminated by blood or sexual fluids) (IARC 1996, 2012). The most common detection methods have been based on detecting anti-HIV-1 antibodies by enzyme-linked immunosorbent assay, with confirmation by laboratory-based Western blot immunoassay or immunofluorescence assay for anti-HIV-1 antibodies (CDC 1989). Anti-HIV-1 antibodies typically cannot be detected by these methods until one to three months after infection (Hecht *et al.* 2011). Several more rapid and sensitive methods have been developed to screen for and confirm the presence of anti-HIV-1 antibodies, HIV-1 protein, and HIV-1 RNA in the blood. Some RNA-based detection methods also can measure HIV-1 in dried blood samples (Smit *et al.* 2014).

Exposure

A significant number of people living in the United States are infected with HIV-1. The current number is about 1.2 million, of whom an estimated 13% are unaware of their infection status (CDC 2015b). It is estimated that about 50,000 new HIV-1 infections occur in the

United States each year. Although the incidence of new HIV-1 infections has remained stable over recent years, it varies considerably by risk group. Gay, bisexual, and other men who have sex with men, particularly young African American men, are most likely to be newly infected with HIV-1 (CDC 2012, 2015b).

AIDS typically results from long-term untreated HIV-1 infection. Approximately 65% of people newly diagnosed with HIV-1 remain untreated (the proportion varying by state of residence), accounting for 90% of new AIDS cases (CDC 2015c). About 1.2 million people in the United States have been diagnosed with AIDS since the start of the epidemic in 1981 (CDC 2015b). In 2013, 47,350 people were newly diagnosed with HIV-1 and 26,700 with AIDS. Since the start of the epidemic, about 660,000 people diagnosed with AIDS have died.

Transmission

HIV-1 is transmitted from one individual to another primarily during sexual activity (oral, anal, or vaginal), when HIV-1 in infected sexual fluids crosses mucous membranes to enter the bloodstream. Infection can also occur by direct blood-to-blood transmission, especially in certain populations, primarily through sharing of needles by injection drug users or, more rarely, by transmission through the skin, such as via needlestick injuries, or by the transfusion of infected blood (if the blood supply is not effectively screened for HIV-1) (IARC 2012). Because the U.S. blood supply and donated organs and tissues are screened for HIV-1, transmission via blood transfusion or organ transplant is expected to be rare (DHHS 2013). The estimated risk of infection via blood transfusion is about one in 1 million to 1.5 million per transfused unit (American Red Cross 2016). Screening of the U.S. blood supply began with a test for HIV-1/HIV-2 antibody in 1985, and nucleic acid testing for HIV-1 began in 1999.

Contact of nonsexual mucous membranes or broken skin with infected blood or body fluids by healthcare workers or first responders may also increase the risk of HIV-1 transmission (CDC 1987, Ippolito *et al.* 1999, Leiss *et al.* 2006); however, the risk of infection by these routes is estimated to be less than 1% (Cardo *et al.* 1997). Transmission of HIV-1 from mothers to children occurs *in utero,* through infection of the child's mucous membranes during birth, or through breast milk.

The two primary behavioral risk factors for HIV-1 transmission in most developed countries are the practice of unprotected sex, particularly unprotected anal sex, and the sharing of needles used to inject drugs. Additional risk factors for HIV-1 infection include other sexually transmitted infections (such as chlamydia and gonorrhea), which can increase the risk of sexually transmitted HIV-1 infection in part by causing inflammation or rupture of mucous membranes in the vagina, vulva, penis, or anus. However, treatment or prevention of other sexually transmitted diseases does not always result in decreased HIV-1 infection rates (as reviewed by Ng *et al.* 2011). Other risk factors include circumcision and hormonal, immune, and genetic factors (IARC 1996, 2012).

Diseases (Non-Cancer), Prevention, and Treatment

The World Health Organization (WHO 2007) classifies four clinical stages of infection, from primary HIV-1 infection to AIDS. The CDC case definition for AIDS (CDC 1992, 1999) includes the presence of over 20 AIDS-associated infections or related conditions or a CD4 cell count in the blood of less than 200/μL, resulting in impairment of immune function (CDC 2015d). The most common non-cancer diseases associated with HIV-1 infection are those that most commonly occur in people with impaired immune systems. These include the fungal infections candidiasis, pneumocystis pneumonia, histoplasmosis, and cryptococcosis; the bacterial infections tuberculosis and mycobacterium avium complex; and the parasitic infections toxoplasmosis and cryptosporidiosis. A number of AIDS-related diseases are caused by viruses (such as cytomegalovirus and the cancer-causing viruses KSHV, EBV, and HPV, as discussed above) (IARC 1996, 2012, CDC 2015d). Some chronic conditions that are more common among HIV-1-infected than noninfected people (such as HIV-1-associated kidney disease) may result in part from long-term treatment with antiretroviral drugs, rather than from HIV-1 infection itself (Feeney and Mallon 2011).

With respect to prevention, behavioral risk-reduction strategies include education about safer sex practices (abstinence, consistent condom use, and testing for HIV-1 status), education about the risk of infection from contact of mucous membranes or broken skin with infected fresh blood, and the use of clean needles, particularly among high-risk populations, including sex workers, injection drug users, and infected pregnant mothers (CDC 2015a).

Effective screening of the blood supply and increased implementation of HIV-1 testing programs using rapid tests have reduced infection rates (CDC 2006). Starting short-term antiretroviral therapy soon after a high-risk exposure can prevent the establishment of HIV-1 infection, and pre-exposure treatment is now recommended for specific high-risk populations (CDC 2014). Early initiation of HIV-1 treatment has been shown to reduce the risk of transmitting HIV-1 to an uninfected partner by 96% (CDC 2016b). The risk of mother-to-infant HIV-1 transmission has been greatly reduced by treatment of the mother with antiretroviral drugs beginning before labor and continuing through breastfeeding, as well as by treatment of the infant immediately after birth and for up to 14 weeks among breastfed infants (Newell and Thorne 2004, UNAIDS 2013). Transmission has also been shown to be reduced by Caesarean delivery (European Mode of Delivery Collaboration 1999).

Treatment to reduce the viral load of HIV-1 consists of five main classes of antiretroviral drugs: fusion or entry inhibitors, integrase inhibitors, protease inhibitors, nucleoside/nucleotide reverse-transcriptase inhibitors, and non-nucleoside reverse-transcriptase inhibitors, which are designed to block various steps in the HIV-1 replication cycle (NIAID 2013). Combinations of these drugs (e.g., protease inhibitors and nucleoside reverse-transcriptase inhibitors) (HAART, or cART) are now incorporated into standard treatment guidelines (e.g., DHHS 2015).

A substantial international effort to develop an effective vaccine for HIV-1 has proved challenging (Wang *et al.* 2015), and no prophylactic or therapeutic vaccine is currently available. The National Institute of Allergy and Infectious Diseases Web site provides updated information on HIV vaccine research (NIAID 2015).

Regulations

Bureau of Prisons (BOP)

The BOP manages infectious diseases in the confined environment of a correctional setting through a comprehensive approach that includes HIV-1 testing.

The BOP may place an inmate who tests positive for HIV-1 in controlled housing status when there is reliable evidence that the inmate may engage in conduct posing a health risk to another person.

Victims of severe forms of human trafficking in federal custody shall receive necessary medical care and other assistance, including free optional testing for HIV-1 and other sexually transmitted diseases in cases involving sexual assault or trafficking into the sex industry.

Department of Defense (DoD)

If required by an agreement or local requirements, HIV-1 testing for deployment of contractors authorized to accompany the force in applicable contingency operations must occur within 1 year before deployment. The Combatant Command surgeon should be consulted in all instances of HIV-1 seropositivity before medical clearance for deployment.

Military health system personnel who provide or coordinate medical care for victims of sexual assault under the Sexual Assault Prevention and Response Program are required to consult with the victim, once clinically stable, regarding further healthcare options, including testing, prophylactic

treatment options, and follow-up care for possible exposure to HIV-1 and other sexually transmitted diseases or infections.

Department of Health and Human Services (DHHS)

Designated states under the Substance Abuse Prevention and Treatment Block Grant program (i.e., any state whose rate of cases of AIDS is 10 or more per 100,000 individuals) must make early intervention services for HIV-1 disease, including testing to confirm the presence of the disease, available to individuals undergoing treatment for substance abuse.

Department of Homeland Security (DHS)

Aliens applying for temporary resident status or adjustment from temporary to permanent resident status are required to submit the result of a serologic test for HIV-1 virus.

Any alien inadmissible under Section 212(a)(1)(A)(i) of the Immigration and Nationality Act, as amended by the Immigration Reform and Control Act of 1986, because of HIV-1 infection may be issued a B-1 (business visitor) or B-2 (visitor for pleasure) nonimmigrant visa and be authorized for temporary admission into the United States for a period of 30 days subject to conditions in 8 CFR 4(f)(2).

Department of Housing and Urban Development (HUD)

HUD implements programs (e.g., Housing Opportunities for Persons with AIDS, Shelter Plus Care) designed to provide rental assistance for permanent housing and supportive services (including health care) for low-income individuals with HIV-1/AIDS and homeless persons with disabilities, including HIV-1/AIDS, and their families.

Department of Transportation (DOT)

Infectious substances are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Department of Veterans Affairs (DVA)

For any record maintained in connection with the performance of any program or activity relating to infection with HIV-1, information may be disclosed to a federal, state, or local public health authority charged with protection of the public health under federal or state law, and to which federal or state law requires such disclosure, if a qualified representative of such authority has made a written request for such record pursuant to such law for a purpose authorized by such law.

A physician or professional counselor may disclose information indicating that a patient is infected with HIV-1 to the spouse of the patient or to an individual whom the patient has (during the process of counseling or of HIV-1 testing) identified as being a sexual partner of the patient.

Food and Drug Administration (FDA, an HHS agency)

Since May 2015, 21 CFR 606, 610, 630, 640, and 660 prescribe procedures, including recordkeeping, donor screening and notification, blood and blood component testing, and product labeling, to guard against the spread of HIV-1 through donation of blood, serum, or plasma.

21 CFR 1270 and 1271 prescribe procedures, including donor screening and tissue testing, to ensure that tissues intended for human transplant or other human cells, tissues, and cellular and tissue-based products are free of HIV-1.

21 CFR 864 identifies class designations (Class I, II, or III) of analyte-specific reagents (e.g., analytes intended as components in tests intended for use in the diagnosis of HIV-1/AIDS) that determine the type of premarketing submission or application required for FDA clearance to market.

21 CFR 866 identifies the *in vitro* HIV-1 drug resistance genotype assay (a device intended for use in detecting HIV-1 genomic mutations that confer resistance to specific anti-retroviral drugs, as an aid in monitoring and treating HIV-1 infection) as a Class II medical device with special controls (i.e., a guidance document) requiring premarket notification for FDA clearance to market.

Patient examination and surgeon's gloves must be sampled and tested for leaks and other visual defects to reduce the risk of transmission of HIV-1.

The labeling of over-the-counter vaginal contraceptive and spermicide drug products containing nonoxynol-9 as the active ingredient must contain warnings that these products do not protect against the transmission of HIV-1/AIDS, may increase the risk of getting HIV-1/AIDS from an infected partner, and should not be used by individuals who have HIV-1/AIDS or are at high risk for HIV-1/AIDS.

Health Resources and Services Administration (HRSA, an HHS agency)

The recovery of organs for the purpose of transplantation from individuals infected with HIV-1 was prohibited beginning in 1988. However, an exception to this prohibition has been in place since 2013, when the transplantation of organs from HIV-1-positive donors was approved for recipients who were HIV-1-positive prior to receiving such organs.

Occupational Safety and Health Administration (OSHA)

Comprehensive regulations have been developed for employers to develop and adhere to exposure control plans for bloodborne pathogens.

All work-related needlestick injuries and cuts from sharp objects that are contaminated with another person's blood or other potentially infectious material must be recorded.

First-aid training program trainees must have adequate instruction in the value of universal precautions for preventing infectious diseases.

Public Health Service (PHS, a division of HHS)

Programs or practitioners engaged in opioid treatment of individuals with an opioid agonist treatment medication must provide counseling on preventing exposure to and transmission of HIV-1 disease for each patient admitted or readmitted to maintenance or detoxification treatment.

Serologic testing for HIV-1 is required for aliens over 15 years of age who are applying for immigrant visas; are students, exchange visitors, or other applicants for nonimmigrant visas required by a United States consular authority to have a medical examination; are outside the United States applying for refugee status; or are in the United States applying for adjustment of their status under the immigration statute and regulations.

Guidelines

Centers for Disease Control and Prevention (CDC, an HHS agency)
National Institutes of Health (NIH, an HHS agency)
HIV Medicine Association (HIVMA) of the Infectious Diseases Society of America (IDSA)

The CDC, NIH, and HIVMA have issued federally approved HIV-1/AIDS medical practice guidelines.

Department of Defense (DoD)

DoD Instruction 6485.01 establishes policy, assigns responsibilities, and prescribes procedures for the identification, surveillance, and management of members of the military services infected with HIV-1 and for prevention activities to control transmission of HIV-1.

Department of Health and Human Services (DHHS)

DHHS has issued guidance regarding enrollment of children with disabilities (including HIV-1, AIDS-related complex, or AIDS) in Head Start programs. The guidance includes direction in the event that a child with disabilities presents a problem involving biting or bodily fluids.

DHHS has issued an updated Public Health Service guideline that prescribes donor screening and notification (e.g., recipient informed consent) procedures to guard against transmission of HIV-1 through organ transplants. This guidance also includes a revised set of risk factors for HIV-1 infection to help improve recipient informed consent and prompt more sensitive laboratory testing of donors and recipients when necessary.

Food and Drug Administration (FDA, an HHS agency)

The FDA has issued numerous guidance documents prescribing procedures (e.g., use of standardized labels, abbreviated donor screening questionnaires) for reducing the risk of virus transmission by blood and blood products.

Health Resources and Services Administration (HRSA, an HHS agency)

The Organ Procurement and Transplantation Network prescribes donor screening, recordkeeping, notification, and organ and vessel packaging, labeling, shipping, and storage procedures to guard against the spread of HIV-1 through solid (vascular) organ transplantation.

National Institutes of Health (NIH, an HHS agency)
Centers for Disease Control and Prevention (CDC, an HHS agency)

The NIH and CDC have published criteria for research involving transplantation of HIV-infected donor organs in HIV-positive recipients to (1) ensure that research using organs from HIV-positive donors is conducted under conditions protecting the safety of research participants and the general public and (2) ensure that the results of this research provide a basis for evaluating the safety of solid organ transplantation from HIV-positive donors to HIV-positive recipients.

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Human Papillomaviruses: Some Genital-Mucosal Types

CAS No.: none assigned

Known to be human carcinogens

First listed in the *Eleventh Report on Carcinogens* (2004)

Also known as HPVs

Carcinogenicity

Some human papillomaviruses (HPVs) of the genital-mucosal type are *known to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in humans.

Cancer Studies in Humans

In epidemiological research, numerous case-control studies have consistently reported strong associations between cervical cancer and infection with HPV-16, HPV-18, or "high-risk" HPVs as a class (discussed under Properties, below). Moreover, several case-control studies have provided strong evidence of positive associations between cervical cancer and other individual HPVs, including HPV types 31, 33, 35, 39, 45, 51, 52, 58, and 59 (Muñoz 2000). Cohort studies have demonstrated that infection with HPV-16 or with high-risk HPVs as a class occurs before the development of high-grade cervical intraepithelial neoplasia (CIN), which is thought to be a precursor of invasive cancer. The evidence from cohort studies is weaker for individual high-risk viruses, possibly because they are less common; among these, the evidence for an association with cervical cancer appears to be strongest for HPV-18 (NTP 2003). It is unlikely that the association between HPV infection and cervical cancer is due to other factors that could increase the risk of cancer, because many studies included these factors in their analysis, and because of the large magnitude of the odds ratios estimated in the case-control studies. Thus, these studies demonstrate that some genital-mucosal HPVs cause cervical cancer. In addition to the association with cervical cancer, there is strong evidence that HPV-16 infection is associated with other anogenital cancers, especially cancer of the vulva (NTP 2003). Evidence also suggests associations between HPV infection and some cancers of the head and neck and, especially, the soft palate (oropharynx), tonsils, and back of the tongue and throat (NTP 2003).

Based on testing of tissue specimens from more than 1,000 invasive malignant cervical tumors from women from 22 countries (collected for the International Biological Study of Cervical Cancer), it was estimated that HPV is present in 99.7% of all malignant cervical tumors, suggesting that HPV infection may be necessary for development of cervical cancer (Walboomers *et al.* 1999). Nonetheless, not all individuals infected with HPV develop cervical cancer. Most HPV infections (about 70%) clear within one to two years, and thus confer little risk of cancer. The specific risk factor for cervical cancer appears to be persistent infection with HPV-16 or other high-risk HPVs. Whether HPV infections persist probably depends both on viral characteristics, such as greater persistence of specific HPV types or variants, and on characteristics of the patient, such as sex-hormone levels, smoking behavior, or immune-system status.

Since human papillomaviruses (some genital-mucosal types) were listed in the *Eleventh Report on Carcinogens,* numerous human cancer studies on HPVs have been published. The International Agency for Research on Cancer concluded that HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 were carcinogenic in humans based on sufficient evidence for the carcinogenicity of HPV-16 in the cervix, vulva, vagina, penis, anus, oral cavity, and oropharynx and sufficient evidence for the carcinogenicity of HPV types 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 in the cervix. IARC also concluded that there was limited evidence for the carcinogenicity of HPV types 6, 11, and 18 in the vulva, penis, and anus; HPV types 6, 11, 16, and 18 in the larynx; HPV-18 in the vagina; and HPV-16 in the periungual skin (the skin around the fingernails or toenails) (IARC 2007)

Cancer Studies in Experimental Animals

Because HPV infections are specific to humans, experimental animals cannot be infected with them. Many studies have investigated the carcinogenicity of various animal papillomaviruses both in their natural host species and in other species. Studies in monkeys, cattle, rabbits, and sheep have shown that animal papillomaviruses cause cancer in their natural hosts. Studies in transgenic mice carrying HPV genes demonstrated that HPV proteins play a role in the development of abnormal tissue growth (dysplasia) and progression to tumor formation. Transgenic mice expressing some HPV type 16 or 18 genes and producing the corresponding viral proteins developed tumors of the cervix and other tissues (Arbeit *et al.* 1994, Comerford *et al.* 1995).

Studies on Mechanisms of Carcinogenesis

Infection with high-risk HPVs is associated with chromosomal aberrations, including abnormal centrosome numbers, chromosomal imbalances at specific chromosomal regions, and changes in chromosome number, including tetrasomy and other types of aneuploidy.

HPV can integrate into the DNA of the host cell and can immortalize and transform cells, enabling them to proliferate and form tumors. Most studies on the mechanisms of HPV carcinogenesis have investigated HPV-16 and HPV-18. HPV types 16, 18, 31, and 33 have been shown to transform cells, types 16, 18, and 31 to immortalize cells, and types 16 and 18 to produce proteins that bind to regulatory proteins of the host cell. The HPV proteins E2 and E5 and the long control region of the HPV genome (discussed under Properties, below) play a role in HPV-induced cell transformation. However, the HPV proteins primarily responsible for immortalization and transformation are E6 and E7, as shown in studies with human and rodent cell cultures. Studies with transgenic mice expressing the E6 or E7 gene further support the notion that the E6 and E7 proteins are important

in HPV-associated neoplasia. Both the E6 and E7 proteins alter the pathways that regulate tissue growth, by interfering with growth receptors or growth factors; production of cytokines has been shown to be altered in cells infected with HPV-16. The E6 protein increases degradation of the p53 tumor-suppressor protein, thereby interfering with apoptosis. The E7 protein disrupts complexes of the transcription factor E2F with the tumor-suppressor protein pRb and related proteins involved in control of the cell cycle and causes their degradation, altering control of transcription and progression of the cell cycle. The E7 protein has been shown to cause abnormal synthesis and duplication of centrosomes, resulting in abnormal mitotic division.

Properties

HPVs of the genital-mucosal type are DNA viruses that infect the genital skin and genital and non-genital mucosa, sometimes causing genital warts or cervical abnormalities. They are members of the family Papillomaviridae, which consists of species-specific non-enveloped viruses that infect the squamous epithelium of the skin and mucosal membranes of animals. More than 100 different HPVs had been identified by 2004, including viruses that cause skin warts as well as the genital-mucosal type (Howley and Lowy 2001). The over 40 genital-mucosal HPVs have been classified as either "high risk" or "low risk"; high-risk viruses have been associated with cervical cancer in human epidemiological studies, whereas low-risk viruses have been associated with genital warts or low-grade CIN (abnormal tissue growth in the cervical epithelium that is unlikely to progress to cancer). Most studies have considered HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 to be high-risk viruses; some studies also include other HPVs, most notably HPV-66. Classification of HPVs is based also on phylogenetic and mechanistic considerations. Most high-risk viruses have DNA sequences highly similar to those of either HPV-16 or HPV-18, suggesting that they are closely related to these types. Studies on the mechanisms of carcinogenesis have shown that high-risk but not low-risk viruses immortalize human keratinocytes (skin cells), interact with the tumor-suppressor proteins pRb and p53, and cause chromosomal aberrations. However, most mechanistic studies have evaluated only a few HPVs, the majority focusing on HPV-16 or HPV-18 and a few on HPV-31 or HPV-33.

HPVs are small (about 52 to 55 nm in diameter), consisting of about 8,000 base pairs of covalently closed, double-stranded DNA. The viral genome consists of a series of open reading frames, each of which is a DNA sequence that codes for an HPV protein, and a long control region, which contains elements that regulate DNA replication and protein synthesis. Productive infection of cells (leading to replication of the virus) is linked to their stages of differentiation. Viral replication can be divided into early and late stages, which occur in cells at different stages of differentiation. Early stages of replication (including attachment of the virus to the cell, entry and uncoating, early gene expression, protein production, and DNA replication) occur in basal cells. These cells are the youngest, least differentiated cells and are located in the lower layers of the epithelium; they are the only dividing cells in the squamous epithelium. Late stages of viral replication, which include the events leading to production of viral particles (late gene expression, production of capsid proteins, vegetative viral DNA replication, and virus assembly and release), occur in the terminally differentiating squamous epithelial cells, which are the oldest, most differentiated cells, in the upper layers of the epithelium. The genes expressed in the early stages of viral replication, designated E1 through E8, are associated with regulation of transcription (e.g., E2) and cellular proliferation (e.g., E6 and E7). The genes expressed in the late stages, designated L1 and L2, encode the two proteins that make up the viral capsid (Howley and Lowy 2001).

Detection

HPV infection is detected by observation of visible lesions or microscopic changes in cells, by detection of HPV DNA, or by detection of antibodies against HPV proteins in the blood. Genital warts (condylomata acuminata) are genital lesions visible to the naked eye; they have a fleshy red appearance and a raised surface that usually extends in papillae. Flat condylomata are flat, nonpapillary lesions; they are more difficult to detect and may be apparent only after swabbing with acetic acid and colposcopic examination, in which they appear as white, flat, shiny lesions. The Papanicolaou (Pap) smear, which involves microscopic examination of stained exfoliated genital cells, detects koilocytosis (the presence of cells with abnormal nuclei and a hollow appearance resulting from collapse of the cell's internal structure) and other signs of CIN; it is used to screen for cervical cancer by detecting high-grade CIN (Trofatter 1997).

The most sensitive and specific method for detecting HPV infection is to test for HPV DNA. DNA testing can be used to detect a broad spectrum of HPV genotypes (Trofatter 1997). Detection of HPV DNA signifies present exposure or persistent infection resulting from a past exposure. The most sensitive HPV DNA tests are (1) those based on the polymerase chain reaction and (2) the hybrid capture assay, which is based on the formation of hybrids between HPV DNA and RNA probes. The most commonly used serological tests for HPV infection measure antibodies (immunoglobulin G) against capsid antigens (most often tested as virus-like particles). Several validation studies have estimated the sensitivity of such serological tests to be approximately 50%, using detection of HPV DNA as a standard (Dillner 2000). Because of their low sensitivity, serological assays are not recommended for diagnostic use, but they are useful for comparison of groups in epidemiological studies, which also commonly use HPV DNA testing. Clinical diagnosis of HPV is most commonly based on the hybrid capture II assay.

Exposure

Studies measuring HPV DNA in tissue samples have shown that a significant number of people in the United States are infected with HPV. The Centers for Disease Control and Prevention (CDC) have estimated that 79 million people in the United States are infected with HPV and the number of new genital HPV cases per year (incidence) to be 14 million (CDC 2014). For most populations of mixed age groups, the prevalence of HPV infection has been estimated at 5% to 15%. The percentage of infected individuals (prevalence) is highest among those who are young and sexually active. U.S. epidemiological studies based on HPV DNA testing indicate that between 25% and 40% of sexually active women aged 15 to 25 are infected (Lowy and Howley 2001). Several follow-up studies reported very high incidences of HPV infection (as detected by HPV DNA testing) among young, sexually active individuals, with three-year cumulative incidences ranging from 43% to 55% (Ho *et al.* 1998, Moscicki *et al.* 2001). HPV-16 appears to be the most prevalent type worldwide (Jastreboff and Cymet 2002). In a study of women aged 18 to 40 with no history of high-grade CIN, among whom the prevalence of HPV was 39%, high-risk HPVs were more common (occurring in 26.7% of women) than low-risk HPVs (occurring in 14.7%) (Peyton *et al.* 2001).

Transmission

Genital-mucosal HPVs are transmitted primarily through sexual contact with infected cervical, vaginal, vulvar, penile, or anal epithelium (IARC 1995). This finding is supported by numerous epidemiological studies demonstrating that HPV infection is associated with behaviors related to sexual activity. Numerous studies of HPV in women have reported a positive association between lifetime number of sex

partners and HPV seropositivity (Sun *et al.* 1999, Silins *et al.* 2000) or the presence of HPV DNA (Franco *et al.* 1995, Kjær *et al.* 1997, Lazcano-Ponce *et al.* 2001). Recent sexual activity, the number of sex partners, frequency of sexual intercourse, and presence of genital warts on sex partners are strong predictors of HPV infection, as indicated by HPV DNA testing (Franco *et al.* 1995, Ho *et al.* 1998). The role of men in carrying HPV infection from one woman to another has been demonstrated in studies showing that cervical cancer is relatively more frequent among wives whose husbands have detectable HPV DNA in their penis or whose husbands have had more extramarital partners (Bosch *et al.* 1996). Penile lesions containing the DNA of high-risk HPVs are frequent among male sex partners of women with CIN (Bleeker *et al.* 2002). There are conflicting reports as to whether HPV is transmitted at birth or perinatally. Infants exposed perinatally to HPV-11, or less commonly to HPV-6, may develop a rare benign tumor of the airway called juvenile-onset recurrent respiratory papillomatosis (Shoultz *et al.* 1997).

Diseases (Non-Cancer), Prevention, and Treatment

HPV infection is one of the most common sexually transmitted diseases, but it appears that the majority of those infected have no symptoms. Among all U.S. men and women aged 15 to 49, the estimated prevalence of HPV infection (based on HPV DNA testing) is 10% to 20%, whereas only 1% have genital warts, and 4% show cellular abnormalities associated with HPV infection (Koutsky 1997). In people infected with HPV, about 90% are asymptomatic, and the infection resolves spontaneously within two years (CDC 2015). Some studies have suggested that low-risk HPV infections are more likely to regress than are high-risk HPV infections (Franco *et al.* 1999, Elfgren *et al.* 2000).

The immune system plays an important role in HPV infection; immunocompromised patients are at increased risk for persistent HPV infection (Lowy and Howley 2001). However, in some individuals, genital-mucosal HPVs infect the cervix, causing lesions of varying severity, including genital warts, low- and high-grade CIN, and invasive cervical cancer (Einstein and Burk 2001). Low-grade CIN (CIN I) is a well-differentiated lesion in which the squamous epithelial cells show alterations characteristic of the cytopathogenic effects of a replicative viral infection, such as the presence of two nuclei or other nuclear abnormalities and koilocytosis. The alterations seen in CIN I are not usually considered to be precursors of cancer. The majority of CIN I lesions are transient and resolve spontaneously, but a small percentage may progress to high-grade CIN or invasive cancer (Jastreboff and Cymet 2002). Both high-risk and low-risk HPVs can cause low-grade CIN (IARC 1995). High-grade CIN (CIN II or III) is characterized by the presence of undifferentiated cells above the lower third of the epithelium (extending into the upper layers) and by nuclear crowding, substantial pleomorphism, loss of tissue organization and cellular polarity, abnormal mitotic figures, and larger numbers of atypical cells than observed in low-grade CIN (IARC 1995). High-grade CIN probably results from persistent HPV infection, and it is more likely than low-grade CIN to progress to invasive cancer. (CIN III is also known as carcinoma *in situ,* or noninvasive cancer.) Microinvasive squamous-cell cervical cancer usually arises from high-grade CIN.

Two HPV vaccines are licensed by the U.S. Food and Drug Administration and recommended by the CDC (CDC 2010). Both vaccines are effective against HPV types 16 and 18, which are responsible for most cervical cancer, and one of the vaccines is also effective against HPV 6 and 11, which cause genital warts. Both vaccines are given in three doses, with the second dose given one to two months after the first and the third dose six months after the first (CDC 2009). In 2015, the CDC Advisory Committee on Immunization Practices recommended a new HPV vaccine that protects against the four HPV types covered by the original vaccine plus five additional cancer-causing types (31, 33, 45, 52, and 58) (Petrosky *et al.* 2015). Treatment of HPV infection depends on the severity of the disease and may involve topical applications, interferon-related therapies, or excision of the lesion via laser methods, surgery, or cryotherapy.

Regulations

No specific regulations or guidelines relevant to reduction of exposure to HPVs were identified.

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Human T-Cell Lymphotropic Virus Type 1

CAS No.: none assigned

Known to be a human carcinogen

Also known as HTLV-1

Carcinogenicity

Human T-cell lymphotropic virus type 1 (HTLV-1) is *known to be a human carcinogen* based on sufficient evidence from studies in humans. This conclusion is based on evidence from epidemiological and molecular studies, which show that HTLV-1 causes adult T-cell leukemia/lymphoma (ATLL), and on supporting mechanistic data. Infection with HTLV-1 has been shown to precede development of the cancer, and the HTLV-1 DNA is integrated into the genome of the infected cells, at the same location in the host-cell DNA in all of the cancer cells in a given individual (Yoshida *et al.* 1984). In addition, over 90% of ATLL patients are infected with HTLV-1, and cancer-causing (oncogenic) viral proteins are produced in the host cells (IARC 2012).

Cancer Studies in Humans

Epidemiological and molecular studies demonstrate a credible association between HTLV-1 infection and ATLL, which is a rare and aggressive T-cell cancer found most commonly in areas where HTLV-1 is endemic, such as Japan, the Caribbean, and the Middle East. Infection with HTLV-1 is one of the diagnostic criteria for ATLL. The original link between HTLV-1 infection and ATLL came from case reports and case-series studies that found consistent evidence of HTLV-1 infection in ATLL cases; over 550 HTLV-1-associated cases of ATLL were reported in case-series studies published between 1985 and 2005 (IARC 1996, 2012). In addition, eight cohort studies were identified, including six studies in Japan (IARC 2012), one study in the United States (Biswas *et al.* 2010), and one study in Israel (Stienlauf *et al.* 2013). The findings of these studies suggest a greater risk of disease and mortality in male than in female HTLV-1 carriers in Japan, but this might not be true for other populations. In four nested case-control studies in HTLV-1 cohorts (Hisada *et al.* 1998a,b, Arisawa *et al.* 2002, Okayama *et al.* 2004), the risk of developing ATLL was greater with higher proviral load (the percentage of circulating CD4 T cells with integrated viral DNA) or higher anti-HTLV-1 antibody levels. Prospective studies and detection of a monoclonal insertion site of HTLV-1 DNA in tumors indicate that infection precedes diagnosis of ATLL.

Studies on Mechanisms of Carcinogenesis

Studies of human cells demonstrate that a key HTLV-1 protein, Tax, can immortalize T cells (enabling them to proliferate indefinitely) both *in vitro* and in immunodeficient mice in the absence of other viral products (IARC 2012). Tax causes cancer by affecting several oncogenic pathways. It interacts with host-cell proteins (specifically, the NF-κB family of DNA transcription factors), leading to increased production of interleukin 2 (IL-2), its receptor, and interleukin 6 (IL-6) (Currer *et al.* 2012). IL-2 is involved in T-cell proliferation, growth, and survival, and IL-6 is involved in inflammation responses and regulation of metabolic, regenerative, and neural processes. Tax also inhibits DNA repair and triggers genetic instability in host cells (Currer *et al.* 2012). Tax is highly immunogenic (able to provoke an immune response) and is normally held in check by the host's immune system. However, in individuals with a weakened immune response, Tax can initiate and promote cancer. Once cancer has been induced, production of Tax may be reduced or eliminated as a result of changes in the *Tax* gene; however, another HTLV-1 viral protein, HTLV-1 bZIP factor (HBZ), and oncogenic mutations in the host cell's DNA, such as mutations of the $p16^{INK4A}$ and *p53* tumor-suppressor genes, play a role in the continued proliferation of the cancer cells (NTP 2016).

Biological Properties

HTLV-1 is an enveloped single-stranded RNA (ssRNA) delta-type retrovirus (an RNA virus that can make a DNA copy of its genome) of the subfamily Oncovirinae; it was originally found in cells from a patient diagnosed with T-cell lymphoma (IARC 1996, 2012, Jacobson and Massoud 2013). It can integrate into the DNA of CD4 T lymphocytes (helper T cells, which are white blood cells involved in immune function). The three main viral genes are *gag*, which encodes matrix and capsid proteins, *env*, which encodes the envelope proteins, and *pol*, which encodes the enzymes reverse transcriptase (which enables HTLV-1 to make a DNA copy of its genome), integrase (which enables integration of the DNA copy into the host genome), and protease (which breaks down proteins). Single-protein genes encode the regulatory proteins Tax and Rex and the accessory proteins p12, p13, p30, and HBZ. Viral gene expression is controlled by promoters and enhancers in the two long terminal repeat regions (regions at the boundaries of the proviral DNA that contain sequences regulating protein production).

The HTLV-1 virus particle is immunogenic, so active viral production will elicit a cytotoxic T-cell anti-HTLV-1 immune response (Cook *et al.* 2013, Carpentier *et al.* 2015), which is thought to be responsible for controlling viral load. During the latent phase (when the virus is not replicating), Tax promotes host-cell proliferation. However, Tax itself is immunogenic; for a latent infection to be maintained, production of Tax is suppressed, and host-cell proliferation is maintained by HBZ, which is less immunogenic than Tax, allowing proliferation of latently infected cells.

Detection

HTLV-1 is rarely detected free in bodily fluids, but is found in peripheral mononuclear blood cells in breast milk, blood, semen, and cerebrospinal fluid (IARC 1996, Carpentier *et al.* 2015, Schafer *et al.* 2015). Detection is most commonly through measurement of anti-HTLV-1 antibodies, but can also involve measurement of viral RNA or DNA in peripheral mononuclear blood cells (allowing measurement of proviral load) or *in vitro* culture of the virus. Initial anti-HTLV-1 antibody screening tests are performed by several different methods, and specimens with positive results are further tested in a confirmatory laboratory-based Western blot immunoassay, polymerase chain reaction amplification, immunofluorescence assay, or

recombinant immunoblot assay (methods for quantitative measurement of antigens) (IARC 1996, Sabino *et al.* 1999). Recombinant immunoblot assays are the most accurate, yielding fewer indeterminate results. The percentage of cells infected with HTLV-1 is determined primarily by the cytotoxic T-cell response against HTLV-1-infected cells and varies widely among infected individuals (Cook *et al.* 2013).

Exposure

Studies measuring antibodies to HTLV-1 have shown that a significant number of people in the United States are exposed to HTLV-1. The number of HTLV-1-infected persons in the United States has been estimated to range from 90,000 to 100,000 persons, based on available U.S. studies, which looked primarily at blood donors and injection drug users and took age, sex, and estimates of worldwide prevalence rates into consideration (Gessain and Cassar 2012). A detailed study of HTLV-1 prevalence in the United States conducted from 2000 to 2009 reported a seroprevalence of 0.0051% (5.1 cases per 100,000) among blood donors in the United States (Chang *et al.* 2014, Cook and Taylor 2014). Previous studies reported U.S. HTLV-1 seroprevalence ranging from 0.009% to 0.025% (Williams *et al.* 1988, Murphy *et al.* 1999, Poiesz *et al.* 2001). However, data from blood-donor studies most likely underestimate the prevalence in the general population, because the donors might be healthier as a group.

Transmission

Transmission of HTLV-1 requires cell-to-cell contact, as the virus is unstable outside of cells. The three main transmission modes are from mother to child, sexual, and via blood transfusion or organ transplantation (IARC 2012). Since the U.S. blood supply has been screened for HTLV-1 since 1988, the estimated risk of infection via blood transfusion is less than one in two million per transfused unit (American Red Cross 2016). Although solid organs for transplantation are no longer screened for HTLV-1 in the United States (see Regulations and Guidelines), only one U.S. case of HTLV-1-related disease transmission from an infected organ donor (HTLV-1-associated myelopathy) has been identified since organ screening was discontinued in 2009 (Ramanan *et al.* 2014).

HTLV-1 infects T cells, mainly CD4 T cells and, to a lesser extent, CD8 T cells (cytotoxic T cells, which kill damaged or infected cells). Cells involved in blood-cell formation can be infected as well (IARC 1996, 2012, Jacobson and Massoud 2013, Carpentier *et al.* 2015, Schafer *et al.* 2015). HTLV-1 transmission via breastfeeding depends on the proviral load of the mother's breast milk and on breastfeeding duration. Mother-to-child transmission occurs during the perinatal period in less than 5% of cases (Hlela and Bittencourt 2014). Risk factors for sexual transmission include unprotected sex with an infected partner, multiple lifetime sexual partners, and infection with sexually transmitted diseases (IARC 2012). HTLV-1 transmission is efficient via transfusion of cellular blood components and needle-sharing by injection drug users. Although the U.S. Food and Drug Administration prescribes procedures for donor screening and tissue testing to ensure that tissues intended for human transplantation are free of HTLV-1, some vascularized human organs are excluded from testing (see Regulations and Guidelines).

Diseases (Non-Cancer), Prevention, and Treatment

Most HTLV-1-infected individuals are lifelong symptomless carriers (Cook *et al.* 2013); only 2% to 5% of infected people develop diseases related to the virus (Hlela and Bittencourt 2014). In addition to ATLL, HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP, a chronic and progressive inflammatory disease of the central nervous system) is the most common clinical manifestation of HTLV-1 (Fuzii *et al.* 2014). Other diseases associated with HTLV-1 include HTLV-1 uveitis (eye inflammation) (Kamoi and Mochizuki 2012) and infective dermatitis (Hlela and Bittencourt 2014).

Prevention involves reducing transmission of HTLV-1 via breastfeeding, sexual transmission, and blood transfusion (McKendall 2014). Prenatal screening for HTLV-1 and counseling of seropositive mothers to avoid breastfeeding reduces mother-to-child transmission (Hino 2011, IARC 2012). Following practices that prevent sexually transmitted infections, such as using condoms and not having multiple or anonymous sexual partners, can reduce sexual transmission of HTLV-1 (Yoshimitsu *et al.* 2013). Counseling and education of injection drug users (e.g., implementation of harm-reduction practices) may be effective in reducing HTLV-1 infection among this population (Goncalves *et al.* 2010). Screening of the U.S. blood supply for HTLV-1, which began in 1988 (American Red Cross 2016), has reduced the risk of transfusion-related transmission (McKendall 2014). There is no vaccine against HTLV-1 (ACS 2015, CDC 2015, FDA 2015), but vaccine development efforts are ongoing (Kuo *et al.* 2011).

Regulations

Department of Transportation (DOT)

Infectious substances are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Food and Drug Administration (FDA, an HHS agency)

21 CFR 606, 610, 630, 640, and 660 prescribe procedures, including recordkeeping, donor screening and notification, blood and blood component testing, and product labeling to guard against the spread of HTLV-1 through donation of blood, serum, or plasma (except testing for source plasma). (The risk of HTLV-1, a highly cell-associated pathogen, is sufficiently mitigated by plasma-derivative manufacturing steps, including validated viral inactivation and removal procedures. These manufacturing procedures therefore obviate the need to test individual donations of source plasma for HTLV-1.)

21 CFR 1271 prescribes procedures, including donor screening and tissue testing, to ensure that tissues intended for human transplant or other human cells, tissues, and cellular and tissue-based products (HCT/Ps) are free of HTLV-1. (However, certain HCT/Ps, such as vascularized human organs for transplantation, are excluded. These are listed in 21 CFR 1271.3(d)(1).)

Occupational Safety and Health Administration (OSHA)

Comprehensive regulations have been developed for employers to develop and adhere to exposure control plans for bloodborne pathogens.

All work-related needlestick injuries and cuts from sharp objects that are contaminated with another person's blood or other potentially infectious material must be recorded.

First-aid training program trainees must have adequate instruction in the value of universal precautions for preventing infectious diseases.

Guidelines

American Society of Transplantation (AST)

The AST has issued guidance for the diagnosis and prevention of HTLV-1 infection from solid (vascular) organ transplantation. AST guidelines advise that HTLV-1 screening may be considered by individual organ procurement organizations with higher HTLV-1 prevalence populations (e.g., a high proportion of immigrants from countries where HTLV-1 is endemic). Their guidelines do not preclude solid organ transplantation from HTLV-1-positive donors, but advise that these donors be used only in extreme circumstances (e.g., life-threatening situations, with informed consent) and that recipients of confirmed or suspected HTLV-1-infected organs be monitored periodically.

Food and Drug Administration (FDA, an HHS agency)

The FDA has issued numerous guidance documents prescribing procedures (e.g., use of standardized labels, abbreviated donor screening questionnaires) for reducing the risk of virus transmission by blood and blood products.

Health Resources and Services Administration (HRSA, an HHS agency)

The Organ Procurement and Transplantation Network (OPTN) prescribes voluntary procedures for HTLV-1 screening, confirmation in potential donors, and reporting of potential HTLV-1 infection to reduce the risk of HTLV-1 transmission through solid organ transplantation. Prior to 2009, the OPTN recommended screening of organs for HLTV-1, but that recommendation was removed effective November 23, 2009, because of the low prevalence of HTLV-1 infection in the United States, the loss of potentially usable organs because of a high rate of false-positive test results, and the lack of an FDA-licensed test for HTLV-1 with a rapid turnaround for analysis of single samples.

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Kaposi Sarcoma-Associated Herpesvirus

CAS No.: none assigned

Known to be a human carcinogen

Also known as KSHV or human herpesvirus 8 (HHV-8)

Carcinogenicity

Kaposi sarcoma-associated herpesvirus (KSHV) is *known to be a human carcinogen* based on sufficient evidence from studies in humans. This conclusion is based on evidence from epidemiological and molecular studies, which show that KSHV causes Kaposi sarcoma, primary effusion lymphoma, and a plasmablastic variant of multicentric Castleman disease, and on supporting mechanistic data. KSHV causes cancer, primarily but not exclusively in people with suppressed immune systems, by coding for protein and RNA products that work together to transform host cells into cancer cells and promote their survival and growth. These viral products are made (through the use of host-cell machinery) both when KSHV is in the lytic phase (destroying the infected cell during replication) and when it is latent (maintaining its DNA in the infected cell without destroying the cell) (Mesri *et al.* 2010, Fukumoto *et al.* 2011). KSHV virus is latent in most infected cells.

Cancer Studies in Humans

The majority of human cancer studies of KSHV have focused on Kaposi sarcoma (a cancer of the cells that line blood or lymph vessels). Kaposi sarcoma has four main subtypes: (1) epidemic, or related to human immunodeficiency virus type 1 (HIV-1) infection, (2) iatrogenic (resulting from medical treatment, such as organ transplants), (3) classic (a slow-growing form found mostly in older men in specific populations, such as in Mediterranean countries or among East Europeans of Jewish descent), and (4) endemic (found in sub-Saharan Africa, mostly among men but also among children).

Other cancer end points, including two rare B-cell non-Hodgkin lymphomas (primary effusion lymphoma and multicentric Castleman disease), also have been linked to KSHV. Primary effusion lymphoma (also called body-cavity-based B-cell lymphoma) arises in a specific type of immune cell (B lymphocytes) and comprises approximately 2% to 4% of HIV-1-related non-Hodgkin lymphomas (Simonelli *et al.* 2003, Sullivan *et al.* 2008). Multicentric Castleman disease also arises in B lymphocytes; it has several histological variants (hyaline

vascular, plasma cell, mixed, and plasmablastic), and KSHV is associated with the plasmablastic variant.

Kaposi Sarcoma

Evidence for an association between KSHV infection and Kaposi sarcoma is based on consistent findings of increased risk in epidemiological studies with different designs and in different populations and on the presence of an exposure-response relationship between the degree of viral infection and the cancer.

Over 90% of Kaposi sarcoma patients are infected with KSHV, and KSHV DNA is found in virtually all Kaposi sarcoma tumors; KSHV is therefore considered to be a prerequisite for diagnosis of this cancer (Chang *et al.* 1994, Mesri *et al.* 2010, Cavallin *et al.* 2014). The association between KSHV and Kaposi sarcoma has been evaluated in about 80 case-control studies and 25 cohort studies or studies of cases and controls identified within the cohorts (IARC 2012, NTP 2016). In the case-control studies, patients with all four types of Kaposi sarcoma were often 10 times and sometimes over 100 times more likely to be infected with KSHV than were individuals without Kaposi sarcoma. In the cohort studies, KSHV-infected individuals were 2 to 16 times more likely to develop Kaposi sarcoma than were uninfected individuals. In some studies, the risk of Kaposi sarcoma increased with increasing viral load of KSHV (Sitas *et al.* 1999, Newton *et al.* 2003a,b, 2006, Albrecht *et al.* 2004).

Most KSHV-infected patients who develop Kaposi sarcoma have immune systems compromised either by HIV-1 infection or as a result of drug treatments after organ or tissue transplants. The timing of infection with HIV-1 may also play a role in development of Kaposi sarcoma. Acquiring HIV-1 infection prior to KSHV infection may increase the risk of epidemic Kaposi sarcoma by 50% to 100%, compared with acquiring HIV-1 infection at the same time as or after KSHV infection. Nevertheless, patients with the classic or endemic forms of Kaposi sarcoma are not suspected of having suppressed immune systems, suggesting that immunosuppression is not required for development of Kaposi sarcoma.

Primary Effusion Lymphoma

Evidence for an association between KSHV infection and primary effusion lymphoma is based on consistent findings from over 115 KSHV-infected patients reported in a large number of case reports, several small case series, and a case-comparison study (IARC 2012, NTP 2016), together with histological confirmation of the tumors' specific morphological and immunological features. These cases of primary effusion lymphoma were identified in different populations, including both HIV-1-positive and HIV-1-negative patients, organ-transplant recipients, and people from areas where the risk of endemic or classic Kaposi sarcoma is known to be high (Dotti *et al.* 1999, Boulanger *et al.* 2008, Testa *et al*. 2010, IARC 2012). In addition, some patients with primary effusion lymphoma also had other KSHV-associated cancers, including Kaposi sarcoma and multicentric Castleman disease. The strong association of KSHV with primary effusion lymphoma led to the adoption of the presence of KSHV infection as a diagnostic criterion for this specific pathologic entity. Primary effusion lymphoma in HIV-1-positive patients exhibits rapid progression with short survival times, whereas progression in HIV-1-negative patients and patients with normal immune responses appears to be much slower (IARC 1997), suggesting that immunosuppression is an important cofactor in KSHV carcinogenicity.

Studies of tumor tissue provide evidence for a causal role of KSHV in primary effusion lymphoma. These studies have shown that primary effusion lymphoma cells contain high levels (50 to 100 copies) of KSHV DNA and have patterns of viral gene products (RNA or protein) very similar to those observed in Kaposi sarcoma. Importantly, primary effusion lymphoma lesions arise from a single KSHV-infected B cell (i.e., are monoclonal), suggesting that KSHV infection precedes tumor growth (Katano *et al.* 1999, Judde *et al.* 2000, Fukumoto *et al.* 2011, IARC 2012, Giffin and Damania 2014).

Multicentric Castleman Disease

Two types of evidence establish a link between KSHV infection and the plasmablastic variant of multicentric Castleman disease. The first consists of nine case-series studies and four case-comparison studies, which provide, although not conclusively, evidence for an association between KSHV infection and all types of multicentric Castleman disease, and the second consists of molecular analysis of this type of the tumor tissue, which demonstrates that the association between KSHV infection and multicentric Castleman disease is strongest for the plasmablastic variant of this disease (NTP 2016). (The plasmablastic variant arises in plasmablasts, which are immature precursors of antibody-producing B lymphocytes.)

KSHV has been detected in about half of all multicentric Castleman disease cases (all variants) reported in the literature, but has only rarely been detected in patients without cancer (i.e., in only 1 of almost 200 controls in the case-comparison studies). It is more common among multicentric Castleman disease patients who are also infected with HIV-1 than in HIV-1-negative patients, suggesting that immunosuppression is important for cancer development (Parravicini *et al.* 1997a, Oksenhendler *et al.* 2002, 2013). Moreover, the level of KSHV DNA in circulating white blood cells or blood plasma is related to the occurrence of symptoms during multicentric Castleman disease flares in HIV-1-positive individuals (Reddy and Mitsuyasu 2011).

Studies characterizing the tumor tissue have shown that plasmablasts in KSHV-associated multicentric Castleman disease have a unique molecular profile and produce a distinctive monotypic form of immunoglobulin M; these plasmablasts are not found in KSHV-negative multicentric Castleman disease (Dupin *et al.* 2000). Therefore, KSHV-associated (plasmablastic) multicentric Castleman disease is now recognized as an entity distinct from other forms of multicentric Castleman disease; it is classified by the World Health Organization as "a large B-cell lymphoma arising in HHV8- [KSHV-] associated multicentric Castleman disease" (IARC 2008). In KSHV-associated multicentric Castleman disease cells, it appears that KSHV proteins are produced both when the virus is latent and when it is replicating. These proteins include a virally encoded interleukin 6, which stimulates proliferation of mature B lymphocytes and causes inflammation, and thus may play a role in carcinogenicity (Aoki *et al.* 2001, Burbelo *et al.* 2010, Fukumoto *et al.* 2011, Giffin and Damania 2014).

Studies on Mechanisms of Carcinogenesis

KSHV-associated cancer develops through a complex process that involves interactions among many viral, host, and environmental factors (Fukumoto *et al.* 2011, Mesri *et al.* 2014). Both *in vitro* and animal models have been developed that accurately reproduce many features observed in KSHV-associated cancer (An *et al.* 2006, Mutlu *et al.* 2007, Mesri and Cesarman 2011, Ashlock *et al.* 2014, Dittmer *et al.* 2015); however, not all aspects of viral infection and transformation into cancer cells are well understood (Fukumoto *et al.* 2011, Giffin and Damania 2014).

In an immune-compromised individual, KSHV-infected cells escape recognition and destruction by the immune system and are able to produce cancer-causing viral RNA or proteins (Cavallin *et al.* 2014, Mesri *et al.* 2014). Viral products made during the lytic phase mimic or disrupt host cytokine signals (communication among cells), creat-

ing conditions that promote tumor growth, proliferation of latently infected cells, development of blood vessels, inflammation, and evasion or alteration of the immune response and antiviral response (Mesri *et al.* 2010, Wen and Damania 2010, Fukumoto *et al.* 2011, Cavallin *et al.* 2014). In addition, some latently infected cells are less likely to provoke an immune response and may progressively transform into cancer cells through inhibition of programmed cell death (apoptosis) and maintenance of viral latency (see NTP 2016).

Biological Properties

KSHV is an enveloped double-stranded DNA gamma-2 herpesvirus (rhadinovirus) that was first identified in humans in 1994 in association with acquired immunodeficiency syndrome (AIDS) (Chang *et al.* 1994, IARC 1997, Fukumoto *et al.* 2011). A lipid membrane envelope surrounds a layer of viral proteins, which encloses a viral capsid (protein shell) and a linear 165-kb genome (IARC 1997, 2012, Fukumoto *et al.* 2011, Giffin and Damania 2014). KSHV infects many types of cells, including both immune cells (B lymphocytes, dendritic cells, and monocytes) and non-immune cells (keratinocytes, fibroblasts, and prostate cells) (IARC 1997, 2012, Fukumoto *et al.* 2011, Campbell *et al.* 2014, Giffin and Damania 2014). Certain immune cells ($CD19^+$ B lymphocytes) are a long-term reservoir for the latent virus. KSHV glycoproteins bind to several host-cell receptors and initiate viral entry by an inward folding of the host cell membrane to form a small capsule within the cell that contains the virus attached to its receptor (Giffin and Damania 2014). The virus can reproduce through the lytic cycle, which destroys the infected cell, or can remain latent as a viral episome, consisting of circular DNA separate from the infected cell's chromosomes, which can use the cell's machinery to replicate along with the infected cell's own DNA.

Detection

KSHV is detected most commonly by measurement of anti-KSHV antibodies, and also by detection of DNA and viral proteins in tissues (Parravicini *et al.* 1997b, 2000, Fukumoto *et al.* 2011, Bhutani *et al.* 2015, Xu *et al.* 2015). Tests are available for detecting antibodies to proteins produced in the latent and lytic phases. These tests vary in sensitivity and specificity, but have generally improved over time. Different viral proteins are made during different phases of the viral life cycle (latent or lytic), and an individual's antibody response to these proteins varies, which makes it difficult to compare the prevalence of KSHV in different populations (IARC 1997, 2012, Fukumoto *et al.* 2011). Viral DNA can be detected in tumor tissue by polymerase chain reaction (IARC 1997, Fukumoto *et al.* 2011, Campbell *et al.* 2014).

Exposure

Prevalence studies measuring antibodies to KSHV in serum have shown that a significant number of people in the United States are infected with KSHV. In the first systematic evaluation of KSHV epidemiology in the U.S. general population (based on tests for KSHV antibodies in serum samples from the Third National Health and Nutrition Examination Survey, 1988–1994), overall prevalence of KSHV antibodies was approximately 7% and was similar in men and women (Engels *et al.* 2007). A previous study of 1,000 U.S. blood donors (sampled in 1994 and 1995) reported estimated prevalence of KSHV antibodies ranging from 0.5% to 5% (Pellett *et al.* 2003, IARC 2012). KSHV prevalence rates appear to vary widely among different populations, from 2% to 3% in northern Europe to over 50% in some sub-Saharan African populations (IARC 2012). However, even outside of areas where KSHV is endemic, prevalence rates among men having sex with men have been reported in the range of 30% to 60% for HIV-1-positive men and 20% to 30% for HIV-1-negative men (Martin *et al.* 1998, O'Brien *et al.* 1999, Phillips *et al.* 2008).

Transmission

KSHV is thought to be transmitted primarily via saliva (IARC 2012). The presence of KSHV in peripheral blood suggests that it can also be spread via blood, and transmission has been reported in injection drug users, in transfusion recipients, and from organ-transplant donors to recipients (Barozzi *et al.* 2003, IARC 2012). In populations in which KSHV infection is endemic, it can be transmitted from mother to child, especially among children between the ages of 6 and 10 years, and infection rates increase with age. Risk factors for infection may include contact with infected family members, contact with contaminated water, and, in particular, HIV-1 infection (IARC 2012). There is also some evidence for spouse-to-spouse transmission among heterosexual couples, which appears to be more efficient from female to male than male to female (Dupuy *et al.* 2009).

Factors that increase the risk of HIV-1 infection (e.g., number of sexual partners) also increase the risk of infection with KSHV (Smith *et al.* 1999, Engels *et al.* 2007, IARC 2012), and orogenital sex has been shown to be significantly correlated with development of KSHV antibodies in men who have sex with men (Dukers *et al.* 2000). It is unclear whether KSHV is sexually transmitted in heterosexuals. It has also been suggested that application of virus-carrying saliva to the sites of insect bites (to relieve itching) could facilitate the transmission of KSHV (Coluzzi *et al.* 2003, Amodio *et al.* 2011).

Diseases (Non-Cancer), Prevention, and Treatment

Most otherwise healthy individuals who are infected with KSHV show no symptoms (DHHS 2013a, ACS 2014, NCI 2014). There are very few reports of primary infection with KSHV. Symptoms associated with initial KSHV infection include fever, a measles-like skin rash, diarrhea, fatigue, swollen lymph nodes, enlarged spleen, blood-cell deficiencies, and bone-marrow failure with an excess of B lymphocytes (Luppi *et al.* 2000a, Wang *et al.* 2001, Andreoni *et al.* 2002). Active KSHV infection may be associated with fever, skin rash, and hepatitis (Luppi *et al.* 2000b). There is conflicting evidence regarding suggested associations of KSHV infection with actinic keratosis or with the autoimmune skin diseases pemphigus vulgaris and pemphigus foliaceus (Ablashi *et al.* 2002). KSHV has been found in inflammatory cells in isolated cases of interstitial pneumonitis (an autoimmune-related lung disease), in sarcoid tissue (lesions formed in sarcoidosis, an inflammatory disease), and in histocytic necrotizing lymphadenitis (a lymph-node disorder), but a causal role for KSHV in these diseases has not been established.

Because KSHV transmission is associated with KSHV shedding in saliva and occasional shedding in genital secretions, avoiding salivary exposure (e.g., via kissing or sharing food, drink, or toothbrushes) and following safe sexual practices may theoretically prevent transmission (Chang-Moore Laboratory 2009, DHHS 2013a,b). Some drugs have been reported to reduce or inhibit KSHV shedding; however, no FDA-approved drugs currently exist for treatment of KSHV infection. Highly active antiretroviral therapy was associated with an 89% decrease in KSHV shedding frequency (Cattamanchi *et al.* 2011). There is no vaccine against KSHV, but limited vaccine development efforts are ongoing (Wu *et al.* 2012, ACS 2014).

Regulations

Department of Transportation (DOT)

Infectious substances are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Occupational Safety and Health Administration (OSHA)

Comprehensive regulations have been developed for employers to develop and adhere to exposure control plans for bloodborne pathogens.

All work-related needlestick injuries and cuts from sharp objects that are contaminated with another person's blood or other potentially infectious material must be recorded.

First-aid training program trainees must have adequate instruction in the value of universal precautions for preventing infectious diseases.

Guidelines

American Society of Transplantation (AST)

The AST has issued guidance for the diagnosis, prevention, and treatment of KSHV infection after solid (vascular) organ transplantation. AST guidelines do not specifically prohibit solid organ transplantation because of KSHV seropositivity in either the donor or the recipient. They do advise that serologic screening be considered for donors and recipients from geographic regions with high rates of KSHV infection.

Food and Drug Administration (FDA, an HHS agency)

The FDA has issued numerous guidance documents prescribing procedures (e.g., use of standardized labels, abbreviated donor screening questionnaires) for reducing the risk of virus transmission by blood and blood products.

Health Resources and Services Administration (HRSA, an HHS agency)

KSHV infection is included as part of a list of potential donor-derived disease transmission events (PDDTE) reported through 2010 in the Organ Procurement and Transplantation Network's (OPTN) guidance for reporting PDDTE; however, these guidelines do not specifically prohibit solid organ transplantation because of KSHV seropositivity in the donor.

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Merkel Cell Polyomavirus

CAS No.: none assigned

Known to be a human carcinogen

Also known as MCV or MCPyV

Carcinogenicity

Merkel cell polyomavirus (MCV) is *known to be a human carcinogen* based on sufficient evidence from studies in humans. This conclusion is based on evidence from epidemiological, clinical, and molecular studies, which show that MCV causes Merkel cell carcinoma, and on supporting mechanistic data. MCV causes cancer by integration of the viral DNA into the host-cell genome and expression of two MCV proteins: small T (sT) antigen and a mutated form of large T (LT) antigen. Only the mutated, integrated form of MCV is associated with carcinogenicity. Merkel cell carcinoma is more common in people infected with human immunodeficiency virus type 1 (HIV-1) and in organ-transplant patients than in people with normal immune function, suggesting that immunosuppression is an important cofactor in MCV carcinogenesis.

Cancer Studies in Humans

Evidence for an association between MCV infection and Merkel cell carcinoma is based on consistent findings of increased risk in epidemiological studies of populations in different geographical areas, a suggestion of a dose-response relationship, and the findings of clinical and molecular studies. Although MCV infection is common, Merkel cell carcinoma is rare; it is a highly aggressive form of skin cancer most common in elderly Caucasian men and in immunosuppressed individuals (such as organ-transplant recipients and people infected with HIV-1) (Engels *et al.* 2002, Schrama *et al.* 2012, Clarke *et al.* 2015).

Three case-control studies (Carter *et al.* 2009, Paulson *et al.* 2010, Viscidi *et al.* 2011) and one nested case-control study (Faust *et al.* 2014) found statistically significant associations between MCV infection (as measured by antibodies against MCV viral molecules) and Merkel cell carcinoma, with odds ratios (ORs) ranging from 4.4 to 63.2. In the nested case-control study (Faust *et al.* 2014), the findings of an association of Merkel cell carcinoma with high levels of anti-MCV antibodies (immunoglobulin G [IgG] antibodies that bind to the virus) or MCV-neutralizing activity (due to antibodies that inhibit infectivity) were mainly limited to women; however, there were few men in this study. The OR was higher among all individuals with high levels of anti-MCV IgG antibodies (OR = 4.3, 95% confidence interval [CI] = 1.3 to 17.4) than among all individuals with any level of MCV IgG antibodies (OR = 2.6, 95% CI = 0.7 to 15.0) (the analysis did not stratify by antibody level), providing limited support for a dose-response relationship. This prospective study also demonstrated a temporal relationship between MCV infection and the onset of Merkel cell carcinoma. Because of the paucity of studies and absence of known risk factors for MCV infection in relation to Merkel cell carcinoma, confounding and chance cannot be ruled out as possible explanations for the results of these epidemiological studies.

Clinical and molecular studies provide strong evidence that MCV has an important causal role in Merkel cell carcinoma (see Studies on Mechanisms of Carcinogenesis). MCV antibody levels were significantly higher in MCV-positive Merkel cell carcinoma patients than in healthy individuals infected with MCV, which suggests that development of Merkel cell carcinoma is preceded by an unusually robust MCV infection (Pastrana *et al.* 2009, Agelli *et al.* 2010). In case-series studies (21 studies with over 850 cases of Merkel cell carcinoma), MCV has been detected in approximately 80% of cases (IARC 2013, NTP 2016), suggesting that there are two forms of Merkel cell carcinoma, the majority occurring in MCV-positive individuals and the minority in individuals not infected with MCV (Moore and Chang 2014). However, other studies have suggested that MCV is involved in most or all cases of Merkel cell carcinoma (Carter *et al.* 2009, Rodig *et al.* 2012).

MCV DNA is integrated into the host genome in primary Merkel cell tumors and metastases, and the DNA integration site is the same in all cells of the tumor and its metastases in a given individual. This monoclonal integration of MCV DNA provides strong evidence that viral infection precedes proliferation of the original cancer cell (Feng *et al.* 2008, Laude *et al.* 2010) and that MCV is not an incidental or passenger infection in Merkel cell carcinoma (Kuwamoto 2011, Chang and Moore 2012).

Studies on Mechanisms of Carcinogenesis

MCV-associated cancer is thought to develop through a complex process that involves interactions among many viral, host, and environmental factors. Although MCV infection is common, very few people develop Merkel cell carcinoma (Chang and Moore 2012, IARC 2013). Data from molecular studies have shown that host cells infected with the episomal (non-integrated) form of the virus are not transformed; only the integrated and mutated form of MCV is associated with cancer (Moore and Chang 2014). Exposure to ultraviolet radiation or other environmental mutagens may increase the likelihood of the mutations that transform symptomless viral infection into cancer (Moore and Chang 2010).

The key events in induction of Merkel cell carcinoma by MCV include immunosuppression and immune evasion, monoclonal integration of the MCV genome into the host-cell DNA, mutations causing truncation of the LT antigen, production of T antigens in tumor cells, and impaired regulation of the cell-division cycle and apoptosis (programmed cell death) (Moore and Chang 2010). Truncation of the LT antigen, which prevents the virus from replicating, is a signature feature of all MCV-positive Merkel cell carcinoma. *In vitro* and *in vivo* studies have clearly demonstrated that both the mutated LT antigen and another T antigen (sT) are required for transformation of the host cell into a cancer cell and for proliferation and survival of the cancer cells (Moore and Chang 2010, Shuda *et al.* 2011, Schmitt *et al.* 2012, Borchert *et al.* 2014, Stakaityte *et al.* 2014). Moreover, these antigens are produced only in MCV-infected tumor cells and are found in most Merkel cell carcinoma samples (for more information, see NTP 2016).

Biological Properties

Merkel cell polyomavirus is a very stable non-enveloped DNA virus found in the skin, and it is integrated into the DNA of most Merkel cell tumors (Moore and Chang 2010, Carter *et al*. 2013, Dalianis and Hirsch 2013, IARC 2013, Spurgeon and Lambert 2013, Moens *et al*. 2015). The structure of the MCV is simple, consisting of two capsid (shell) proteins with a circular 5-kb DNA genome that wraps around structural histone (DNA packaging) proteins derived from the host cell (Dalianis and Hirsch 2013, IARC 2013, Spurgeon and Lambert 2013, Moens *et al*. 2015). Once MCV has entered a host cell, its DNA is maintained in a form that allows it either to replicate independently or to integrate into the host cell's DNA for replication (IARC 2013). MCV can exist in either a lytic phase (in which the infected cell is destroyed and viral particles are released) or a latent phase (in which the virus does not replicate). During the latent phase, little viral gene expression occurs, and the virus can evade immune detection.

Detection

MCV infections can be detected in body fluids (blood, saliva, or urine) by the presence of anti-MCV antibodies or by polymerase chain reaction (PCR) amplification of the viral DNA and in tissue (skin, mouth, liver, or Merkel cell tumors) by the presence of viral antigens or DNA (Moore and Chang 2010, Dalianis and Hirsch 2013, IARC 2013, Moens *et al.* 2015). The rates at which MCV DNA has been detected in skin samples have been reported to be up to 28% by standard or nested PCR, 40% by rolling circle DNA amplification, and 100% by real-time or quantitative PCR (IARC 2013). MCV DNA detection rates in the oral cavity have been reported to range from 8.3% to as high as 60%. Some studies have detected MCV DNA at higher rates in the oral-cavity mucous membranes than on the skin, while others have reported the opposite, possibly because of differences in sampling methods (e.g., biopsies vs. surface swabs). MCV DNA found on one area of the skin is genetically identical to MCV found on other areas of skin. The level of anti-MCV antibodies in the blood correlates with viral load on the skin and with active viral shedding.

Exposure

Studies measuring antibodies to MCV have shown that a significant number of people in the United States are infected with MCV. Reported U.S. MCV seroprevalence rates have ranged from 23% to 88% (Carter *et al.* 2009, Kean *et al.* 2009, Pastrana *et al.* 2009, Tolstov *et al.* 2009, 2011, Viscidi *et al.* 2011). Age-specific MCV seroprevalence has been reported to be 20% in children aged 1 to 5 years, 35% to 50% in those under 10 to 15 years old, and 46% to 87.5% in adults (Tolstov *et al.* 2009, Chen *et al.* 2011, Viscidi *et al.* 2011, IARC 2013). MCV may be undetectable in newborns (Gustafsson *et al.* 2012).

Transmission

Transmission of MCV is not fully characterized (IARC 2013). The skin appears to be a primary site of MCV infection, and healthy individuals have been shown to chronically shed MCV DNA from the skin surface (Schowalter *et al.* 2010). Possible transmission through close personal contact via saliva or skin between young siblings and between mothers and their children is suggested by a study in Cameroon, Central Africa (Martel-Jantin *et al.* 2013), and a cross-sectional study of a large rural Chinese population found that poor personal hygiene (e.g., infrequent bathing) may increase the risk of cutaneous transmission of MCV via frequent close contact or shared family environment (Zhang *et al.* 2014). MCV DNA has also been detected in saliva, the mouth, the esophagus, and the colon (Loyo *et al.* 2010) and in nasal mucus samples, tonsils, lung tissue, and secretions in the lower respiratory tract (IARC 2013), suggesting possible transmission via the digestive tract (e.g., fecal-oral transmission) and respiratory tract. Reports of detection of MCV DNA in blood or urine have been inconsistent; a few studies have found MCV at low levels in small percentages of the samples (IARC 2013). Because most adults have MCV antibodies, blood is not expected to play a large role in transmission, and the low levels of MCV reported in urine could be due to contamination from skin during passing of urine. MCV has been detected in urban sewage (Spurgeon and Lambert 2013) and in 85% of environmental surface samples (Foulongne *et al.* 2011, IARC 2013). It is stable at temperatures up to 167°F, suggesting that virus present in the environment may remain viable and be a source of infection.

Diseases (Non-Cancer), Prevention, and Treatment

MCV establishes a chronic lifelong, symptomless infection in a large majority of healthy individuals, but it has not been associated with any disease or symptoms other than Merkel cell carcinoma (IARC 2013). Some treatments of MCV-positive Merkel cell carcinoma target MCV T antigens (Samimi *et al.* 2015). There is no vaccine against MCV (CDC 2015, FDA 2015), but limited vaccine development efforts are ongoing (Pastrana *et al.* 2009, Zeng *et al.* 2012, Gomez *et al.* 2013, Samimi *et al.* 2015).

Regulations

Department of Transportation (DOT)

Infectious substances are considered hazardous materials, and special requirements have been set for marking, labeling, and transporting these materials.

Occupational Safety and Health Administration (OSHA)

First-aid training program trainees must have adequate instruction in the value of universal precautions for preventing infectious diseases.

Guidelines

No specific guidelines relevant to reduction of exposure to MCV were identified.

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