There is substantial evidence that infectious agents play a causal role in a variety of human malignancies. These cancers are surprisingly diverse and include the liver, cervix, stomach, nasopharynx, bladder, and bile duct as well as Kaposi sarcoma (KS) and several lymphomas. In terms of the magnitude of associated cancer cases, the most important oncogenic agents are viruses. These include two hepatotropic viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV) (IARC, 1994a); two herpesviruses, Epstein-Barr virus (EBV) and human herpesvirus 8/KS virus (HHV8) (IARC, 1997); the human papillomaviruses (HPV) (IARC, 1995); and the retrovirus human T-cell lymphotropic virus 1 (HTLV-1) (IARC, 1996). In addition, two chronic parasitic infections, the liver fluke Opisthorchis viverrini and the blood fluke Schistosoma haematobium, have long been thought to be human carcinogens, and more recently a bacterium, Helicobacter pylori, has been implicated (IARC, 1994b) (Table 26–1).

The public health impact of the oncogenic effects of these infections is substantial. It has been estimated that about 15% of all incident cases of cancer worldwide are attributable to infections; this accounts for 23% of all malignancies in economically developing countries and 7% in developed countries (Parkin, 1999). Because these malignancies tend to occur relatively early in life (Doll, 1978) (Fig. 26–1), the impact on year-of-life lost due to cancers caused by oncogenic infections is somewhat greater than for other carcinogenic exposures with similar attributable risk percent.

Table 26–1 summarizes the major oncogenic infections. Several are quite prevalent infections, such as EBV, HPV, and H. pylori. Others are relatively uncommon infections such as HTLV-1, which is microendemic in relatively isolated populations in southern Japan, the Caribbean, South America, and parts of Africa. Endemic infection with O. viverrini is found in parts of Asia and the former Soviet Union, while that of S. haematobium is scattered throughout Africa. The epidemiological patterns of HHV8, HBV, and HCV infections are more variable. These are widely spread throughout the world, generally at a low prevalence but with pockets of relatively high endemicity.

The evaluation of causality for these infectious agents as human carcinogens is difficult given their ubiquitous nature, the substantial length of time between infection and the occurrence of cancer, the nature of cofactors, and the rarity of malignancy among those infected (Evans and Mueller, 1990). That being said, the International Agency for Research on Cancer (IARC) has completed a series of monographs that reviewed the evidence for carcinogenicity for each. The Working Groups concluded that for all of the agents there is “sufficient evidence” for classification as a human carcinogen, with the exception of HHV8, which was classified as “probably carcinogenic” to humans. These judgments have rested heavily on the epidemiological and biomarker evidence.

The agents share several biological characteristics. Each has the capacity to become persistent, creating a carrier state in which periodic or continuing transmission of the agent to new hosts occurs (Ahmed et al., 1996). This can occur as part of the normal life cycle of the agent, such as for herpesviruses EBV and HHV8, which persist as episomes in infected cells. Alternatively, the agent has the capacity to become a conditionally persistent or chronic infection, such as HBV, which can become a chronic infection if not initially cleared by an effective immune response.

For each of these agents, the occurrence of malignancy is a relatively uncommon sequela. This observation underscores the importance of the dynamic host-agent interaction. When host cellular immunity is severely compromised, for example, by induced immunosuppression in organ transplantation or by infection with the human immunodeficiency virus (HIV), the risk of malignancy secondary to the loss of control of latent oncogenic viral infections is substantial (Mueller, 1999). For chronic HBV and HCV infections, interferon (IFN) therapy to enhance cellular immunity sometimes clears the persistent infection and substantially reduces the risk of liver cancer.

In most cases, the host immune competency at the time of infection is important in either clearing the infection, such as for HCV, or generating an adequate cytotoxic T-lymphocyte (CTL) response for setting a favorable host response to control latent infections, such as HPV. Factors that influence the initial host–agent interaction include age and route of infection, gender, and the presence of coinfections and other comorbidity. The general effect of age of infection is reflected in the unusual age-incidence patterns characteristic of these tumors (Fig. 26–1).

A paradigm of cytokine-mediated immune function is useful in this setting (Mossman and Moore, 1991; Lucey et al., 1996; Romagnani, 2000). The paradigm describes two counter-regulating cascades of cytokine responses to specific antigens. Cytokines are secreted by helper T-cells upon presentation of antigens and modulate the resulting immune response to an agent (along with cytokines secreted by other cells). Together they form a complex system of selective stimulation and cross-regulation that results in an immune response to an antigen or family of antigens. “Type 1” (or Th1) responses involve what have long been thought of as cell-mediated immunity, with inflammation and CTL responses. “Type 2” (or Th2) responses induce immunoglobulin (Ig)-based immunity. These two types of T-cell reaction are mutually counter-regulatory, with specific immune responses determined by a relative predominance of either type 1 or type 2 cytokines and effectors (Romagnani, 2000).

Viewing immunity as a spectrum ranging from a predominantly humoral response to a predominately cellular immune response, there is a growing body of evidence linking polarized (likely systemic) or imbalanced responses with a variety of malignancies, autoimmune diseases, and allergic states (Lucey et al., 1996; Kero et al., 2001; Bach, 2002; Simpson et al., 2002). These characterizations of diseases by “type” of immune response have involved in vitro measures of cytokines, such as IL-10, a major type 2 cytokine, or in some cases, serologically detectable products of T-cells, such as sCD30, also a type 2 biomarker (Lucey et al., 1996). A type 1 inflammatory response is typically directed against viral, microbial, and other intracellular infections and is also responsible for delayed-type hypersensitivity. However, excessive type 1 immunity can cause tissue damage and some autoimmune conditions, such as multiple sclerosis and rheumatoid arthritis. In contrast, a type 2 response promotes IgE production and eosinophil function, which are involved in the pathogenesis of allergic inflammation and a variety of atopic conditions.

At birth, the immune system of infants is skewed toward type 2 immunity. The counterbalancing type 1 immunity matures with age, likely due to infection with Th1 stimulatory antigens, such as viruses (Barriere et al., 1996; Kramer et al., 1999). The immune system of children who are sheltered from early contact with viruses may continue to be biased toward type 2 immunity, which promotes the
Table 26-1. Major Human Infection–Associated Malignancies

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>Agent (Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinomas</td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>Schistosoma haematobium (blood fluke)</td>
</tr>
<tr>
<td>Cervical</td>
<td>HPV (papillomavirus)</td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>HBV (hepadnavirus)</td>
</tr>
<tr>
<td>Bile duct</td>
<td>HCV (flavivirus)</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>Opisthorchis viverrini (liver fluke)</td>
</tr>
<tr>
<td>Stomach</td>
<td>Helicobacter pylori (bacterium)</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>HTLV-I (retrovirus)</td>
</tr>
<tr>
<td>Adult T-cell</td>
<td>EBV (herpesvirus)</td>
</tr>
<tr>
<td>Burkitt</td>
<td>EBV (herpesvirus)</td>
</tr>
<tr>
<td>Hodgkin</td>
<td>EBV (herpesvirus)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>HHV8 (herpesvirus)</td>
</tr>
</tbody>
</table>

EBV, Epstein-Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV8, human herpesvirus 8; HPV, human papillomavirus; HTLV-I, human T-cell lymphotropic virus type I.

deviation of asthma and allergic conditions (Cookson and Moffatt, 1997; Shirakawa et al., 1997; Martinez and Holt, 1999). Although the infection-associated malignancies have generally been related to diminished cellular immunity (type I), this may actually be accompanied by immune activation—as seen in HIV infection. Optimally, humans should have a “… well balanced Th1 and Th2 response, suited to the immune challenge” (Berger, 2000).

A common feature of the natural history of all these oncogenic infectious agents is that risk of malignancy is associated with a chronic or increased level of replication, raising the probability of secondary genetic damage to the affected tissues. The mechanisms of oncogenesis are fairly well understood for some infections, whereas for others they are not at all defined. The oncogenic viruses have evolved strategies to interfere with cell cycle control and, in some cases, to induce transformation of infected cells. For example, in HPV-16 (the prototypic high-risk strain) infection, the viral product E6 inactivates the p53 tumor suppressor protein by inducing its rapid degradation. Similarly, the E7 protein binds with the tumor suppressor retinoblastoma protein, resulting in inactivation and degradation. E7 also activates cyclin-dependent kinase 2, which is involved in the transition from the G1 to the S phase of the cell cycle. In HPV-associated cervical cancer, E6 and E7 are usually selectively expressed (Alani and Munger, 1998). Similar viral protein–host gene interactions have been described for the EBV (Spender et al., 1999). In HTLV-I infection, the product of the regulatory gene Tax transactivates a number of host oncogenes while down-regulating the expression of the DNA repair-enzyme gene for β-polymerase (IARC, 1996). HTLV-I, EBV, HBV, HCV, and HHV8 all have evolved mechanisms to activate the NFκB pathway that transcriptionally activates multiple genes that influence cell cycle and enhance the survival of infected cells (Hiscott et al., 2001). The mechanisms by which H. pylori and the oncogenic flukes induce malignancy are not well defined, although a role for chronic inflammation is often cited. Chronic inflammation may also contribute to the oncogenesis of HCV and HBV.

Because the great majority of carriers of these oncogenic infections do not develop subsequent malignancy (or other serious disease), a central problem for the epidemiologist is to define the natural history of infection and identify those factors that are related to the development of cancer. This approach was introduced to cancer epidemiologists by Francis et al. (1979, 1981) who described the natural history of feline leukemia (FeLV), a common retroviral infection among outbred cats in the United States at that time (Fig. 26–2), and noted its similarities to that of HBV infection. With FeLV infection, it is the small proportion of infected cats that are chronically viremic that are
at high risk of developing a FeLV-positive leukemia or lymphoma. The determinants of chronic viremia, as identified by a peripheral blood smear assay, include early age of infection and/or heavy exposure by infectious saliva from multiple viremic cats. The evolution of malignancy occurs over a relatively long period of time. The understanding of the natural history of FeLV infection contributed to the development and implementation of a vaccine program. This model illustrates many of the principles that characterize oncogenic infections of humans.

Natural history research for human oncogenic infections requires informative biomarkers of the agent (such as viral load) and host (such as abnormal antibody pattern) interaction. Interaction with other oncogenic exposures, such as immunosuppressive ultraviolet (UV) light, tobacco use, and unhealthy diet, should be considered. Prospective cohorts of carriers with serial biospecimens provide a rich venue for natural history studies. However, the small number of expected cases, necessitating the identification of intermediary end points, hampers such studies. Such data can be maximized by collaboration among cohorts. Gaining understanding of the natural history of these infections should help identify those carriers who are at high risk of malignancy and guide the development of interventions. This chapter summarizes the biological and epidemiologic features of each of the major oncogenic infections, beginning with the viruses, followed by H. pylori, and with a brief summary of the relevant parasites.

**EPSTEIN-BARR VIRUS**

The EBV is a ubiquitous herpesvirus that infects the great majority of the world's population. EBV establishes latent infection in B-lymphocytes and can induce proliferation of latently infected cells. Although most EBV infections are benign, its oncogenic potential is well documented (Anagnostopoulou and Hummel, 1996). An IARC Working Group on the evaluation of carcinogenic risks to humans concluded that there is sufficient evidence for the carcinogenicity of EBV in the causation of Burkitt lymphoma (BL), Hodgkin lymphoma (HL), nasopharyngeal carcinoma (NPC), immunosuppression-related lymphoma, and sinonasal angiocentric T-cell lymphoma (IARC, 1997).

A member of the gamma-herpesvirus family (gamma-one subgroup), the EBV (also termed human herpesvirus 4) is a linear, double-stranded 172-kb DNA molecule that codes for at least 100 viral proteins (Miller, 1990; Kieff, 1996). Like all herpes viruses, EBV consists of an outer envelope with external, glycoprotein spikes, a viral capsid, and a DNA core. There are two major EBV strains (1 and 2), which differ somewhat in their geographic distribution.

EBV was identified in 1964 in a culture established from a BL tumor (Epstein et al., 1964; Epstein, 1999). It was soon found that B-lymphocytes infected with EBV in vitro become "immortalized," being capable of continual growth. In this process, the cells enlarge into blastoid forms, lose their contact inhibition, and develop new antigens on their surface. It was also determined that lymphoma could be induced in vivo in cotton-top marmosets and owl monkeys by the injection of EBV or EBV-infected lymphocytes (Epstein et al., 1973; Miller, 1974; Miller et al., 1977).

**Natural History of EBV Infection**

The great majority of EBV infections are transmitted by oral contact with virus shed in saliva from an EBV carrier. EBV enters and multiplies in B-lymphocytes via the C3d complement receptor (CD21) (Fingeroth et al., 1984; IARC, 1997). It can also multiply in epithelial cells of the oropharynx, parotid gland, and uterine cervix (Miller et al., 1987). The presence of the virus in cervical secretions (Sixbey et al., 1992; Budman et al., 1992), necessitating the identification of intermediary end points, hampers such studies. Such data can be maximized by collaboration among cohorts. Gaining understanding of the natural history of these infections should help identify those carriers who are at high risk of malignancy and guide the development of interventions. This chapter summarizes the biological and epidemiologic features of each of the major oncogenic infections, beginning with the viruses, followed by H. pylori, and with a brief summary of the relevant parasites.
The epidemiology of EBV infection within a population reflects the prevalence of factors that influence the age of exposure to the virus. In general, most infections occur in childhood. Factors influencing the probability of childhood infection with EBV include the amount of exposure to other children within the household, neighborhood, or in group care settings; the level of hygiene; the sharing and prechewing of food; and perhaps oral contact with adults (Evans and Niederman, 1989). For those individuals who escape childhood infection, the onset of dating and intimate kissing in adolescence is associated with the incidence of EBV infection—with a high probability of IM (Evans and Niederman, 1989).

EBV infection is prevalent throughout the world, even in the most remote tribes along the Amazon (Black et al., 1974). Early seroepidemiologic studies established that the prevalence of infection varied with general socioeconomic conditions (Fig. 26-4). In economically developing populations, infection generally occurs early in life. In Ghana, for example, about 80% of the children are infected by the time they reach 18 months of age (Biggar et al., 1978). Infection typically occurs later in life in economically developed countries where transmission to children is less frequent because of less dense housing, smaller families, and better hygiene. For example, about half of entering American college students in the early 1970s lacked EBV antibody (Evans and Niederman, 1989). With the more recent social changes in the family in the United States including the widespread employment of women, and the increasing use of day care and nursery school for young children (Jamieson et al., 2001), this has likely changed.

EBV Biomarkers

Biomarkers for EBV DNA/RNA and viral-encoded proteins and antibodies have been used in epidemiologic studies of EBV-associated malignancies. For detection of the viral genome fragments in tumor tissue, the techniques that were initially used include the slot and Southern blot (Weiss et al., 1987), polymerase chain reaction (PCR) (Uhara et al., 1990), and in situ hybridization (Anagnostopoulos et al., 1989) assays. Other than the latter, these methods do not distinguish whether any EBV detected is from the neoplastic cells or infiltrating lymphocytes.

What has become commonly used to characterize the EBV-positivity status of a tumor is in situ hybridization to detect the abundant EBERs that are actively transcribed in latently infected cells (Wu et al., 1990). The EBER-1 probes are viewed as the most sensitive in detecting EBV genome in paraffin-embedded tissues and are often combined with in situ hybridization with monoclonal antibodies to test for the presence of the latent viral gene products, such as LMP-1 (Pallesen et al., 1991; Gulley et al., 2002). The clonality of the episomal EBV genome can be determined using Southern blot probes for the EBV terminal repeats (Raab-Traub and Flynn, 1986).

The systemic EBV viral load in peripheral blood mononuclear cells can be quantified by a variety of PCR techniques (Lechowicz et al., 2002). A surprisingly robust new biomarker is cell-free EBV DNA.
measured by PCR in serum or plasma (Mutirangura, 2001; van Esser et al., 2001; Leechowicz et al., 2002). We have found detectable levels in serum specimens collected several years preceding the development of EBV-positive HL (R. Ambinder et al., personal communication).

The quantification of antibody levels (primarily IgG and IgA) against the major EBV antigens, VCA, EA complex (less so EA-R and EA-D), and EBNA complex, has been a major empiric tool in establishing an association with EBV and cancer and providing insight into the virus–host interaction. A schema developed by Rickinson and Kieff (2001) of the relative levels of several antibodies, after primary infection in asymptomatic carriers, and after immune suppression is useful in interpreting antibody profiles (Fig. 26–3). Historically, measurement of EBV antibodies has relied on immunofluorescence (IF)-based tests (Redman and Klein, 1973; Henle et al., 1974). Because the EBV-infected cell lines used in these IF assays may differ in the amount of antigen produced, titer levels from different laboratories may not be comparable but should be internally consistent, as evidenced in de Thé et al. (1978). Assays have been developed to measure anti-EBNA-1 and EBNA-2 (Lennette et al., 1993). Antibodies against other informative antigens include anti-ZEBRA (as a mark of viral reactivation) (Drouet et al., 1999) and EBV-specific DNase, which is predictive of NPC (Chien et al., 2001).

Although enzyme immunoassay (EIA) methods are more commonly used in clinical settings to measure antibodies against the major antigens, such as VCA and EA, they are not widely used in epidemiologic studies, and the comparability of results with those based on IF assays is not always clear. Unfortunately, few laboratories now exist that are able to carry out quantitative determinations of EBV antibodies over the range of antigens in the volume required for epidemiologic studies.

The Major EBV-Associated Malignancies

Burkitt Lymphoma

Burkitt lymphoma (BL) is a malignancy that occurs endemically, primarily among young children, especially boys, in central Africa and New Guinea and sporadically among all ages in other parts of the world. Endemic areas correlate strongly with the presence of holoendemic malaria (Fig. 26–1). The risk of BL is thought to result from the enhanced proliferation of B-lymphocytes by early infection with EBV, interacting with the ongoing mitogenic effect of malaria. In these areas, mainly children are affected, with a peak incidence at about 8 years of age. The jaw and abdominal organs are the most frequent sites of the malignancy (Evans and Mueller, 1997).

Elsewhere, BL is a rare tumor. Approximately one in three of these nonendemic BL cases are EBV-positive, compared to nearly 100% of the endemic tumors (Evans and Mueller, 1997). Of note, BL does occur as an opportunistic malignancy in HIV infection, more commonly in children (Biggar and Rabkin, 1992).

Independent of geographic origin, essentially all BL show one of the three following reciprocal chromosomal translocations involving the long arm of chromosome 8q: these include either 1q4 (75% of the cases), or the short arm of chromosome 2p (9%), or the long arm of chromosome 22q (16%). These translocations have been found consistently in more than 100 established BL cell lines, irrespective of EBV positivity. The translated portion of chromosome 8 includes the c-myc oncogene, which, when translocated adjacent to the heavy chain genes on 14q, or the K light chain gene on 2p, or the D light chain gene on 22q, becomes activated (derepressed) during antibody generation. Alteration of c-myc activity appears to be an essential event in the development of the malignant cell clone whose replication leads to BL (Leder, 1985a, b).

The association of EBV with BL is defined by the demonstration of clonal episomal EBV in tumors, characteristically expressing only EBNA-1 (Klein, 1994). In terms of antibody patterns, a significantly higher prevalence of elevated IgG antibody titers to the VCA, EBNA, and, to a lesser extent, to EA-R, occurs than in age and gender-matched controls from the same area (Henle et al., 1969) (Table 26–2).

The most important epidemiologic study that linked EBV infection with the risk of BL was a prospective study based in a cohort of 42,000 children, aged less than 9 years, in the West Nile district of Uganda, under the auspices of IARC (de Thé et al., 1978). Baseline blood specimens were obtained and stored. Fourteen BL cases occurred in the first 5 years of follow-up, and two additional cases were later identified (Geser et al., 1982). All patients with BL had EBV antibody present in the initial serum sample, taken between 7 and 54 months prior to diagnosis, and 12 of 13 EBV-associated BL cases had pretumor VCA antibody titers as high as, or higher, than any control bled at the same time. The risk for development of BL in children with titers two dilutions or more above the geometric mean titer (standardized for age, sex, and area) of the controls was estimated to be 30 times higher than those with normal levels. In addition, 9 of the 10 confirmed cases, from whom biopsies had been obtained at the time of diagnosis, had detectable EBNA or EBV DNA in the tumor tissue, including one case in whom the pretumor antibody level was normal. Antibodies to other herpesviruses as well as to measles virus were not elevated in the baseline bleedling, indicating a specificity of the EBV association. This study serves as a prototype for establishing the causal association of EBV and other oncogenic infections with subsequent malignancy.

Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) occurs worldwide. These tumors are generally histologically classified as poorly or undifferentiated carcinomas and exhibit substantial infiltration of lymphocytes (Agathanggelou et al., 1995). The highest incidence is in the Far East, primarily in persons of Chinese descent and in related populations, wherever they may migrate (de Thé et al., 1989). Rates differ among various Chinese populations in the same area and are highest among

Table 26–2. Pattern of EBV Biomarkers in Associated Malignancies

<table>
<thead>
<tr>
<th>Marker</th>
<th>Burkitt Lymphoma</th>
<th>Nasopharyngeal Carcinoma</th>
<th>Hodgkin Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCA</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>IgG</td>
<td></td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>IgA</td>
<td></td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>EBNA</td>
<td></td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Early antigen</td>
<td></td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Diffuse</td>
<td></td>
<td>↑ (IgA)</td>
<td>↑</td>
</tr>
<tr>
<td>Restricted</td>
<td>↑*</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>Molecular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clonality</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Latent phenotype</td>
<td>EBNA1+/LMP1+</td>
<td>EBNA1+/LMP1+/LMP2*</td>
<td></td>
</tr>
</tbody>
</table>

EBNA, Epstein-Barr nuclear protein; EBV, Epstein-Barr virus; LMP, latent membrane protein; VCA, viral capsid antigen.

*Predictive of recurrence after treatment.
those from the South. The age-specific incidence among Chinese in Hong Kong and Singapore shows a rising peak from age 20 to 24 to about age 50, then drops off thereafter (Fig. 26–1). This type of age curve suggests an infectious etiology, with exposure occurring early in life (Doll, 1978). In Sweden in the 1980s, by contrast, the rise occurred some two decades later and continued through the end of life (Parkin et al., 1992). In the United States, a trimodal distribution has been noted by Green et al. (1977), who suggest that this may reflect varying etiologies operating in different age groups in a racially mixed population. The epidemiology of NPC is described in detail elsewhere (see Chapter 31).

As in the case of BL, high antibody titers to VCA-IgG mark an association with EBV in NPC (Hene, et al., 1970, 1973). Such high titers are found in more than 90% of NPC patients. Antibody to EA is also elevated, but usually it is the diffuse form (EA-D) (Table 26–2). The level of these antibodies increases as the stages of disease progress (Hene et al., 1977). Mathew et al. (1994) found that of 100 NPC patients with anti-VCA-IgA, 75% had IgG antibodies against ZEBRA, indicative of viral replication. NPC patients also have high antibody levels against EBNA (de Thé et al., 1989). Chatani et al. (1991) have reported that NPC cases have high titers against both EBNA-1 and EBNA-2.

Evidence of EBV in NPC tissue has also been found regularly in biopsies (including carcinoma in situ) by several molecular techniques, and occurs predominantly in epithelial cells, not in the infiltrating lymphocytes (Wolf et al., 1973; Klein et al., 1974; Pagano et al., 1975; Yeung et al., 1993). The viral genome is clonal (Raab-Traub and Flynn, 1986) and expresses a restricted pattern of viral latent proteins, with only EBNA-1, LMP-2A, and inconsistently LMP-1 detected (Fahrus et al., 1988; Hessinger et al., 2004) (Table 26–2). Serologically detectable cell-free EBV DNA can be found at diagnosis, and its return after treatment is predictive of recurrence (Mutirangura, 2001).

A key study that provided strong evidence that EBV plays a primary causal role in NPC was conducted by Pathmanathan et al. (1995). The investigators screened 5362 nasopharyngeal biopsy samples from Malaysia as a means of identifying preinvasive lesions, including dysplasia and carcinoma in situ; they identified 11 biopsies with such lesions that lacked adjacent invasive carcinoma. Of these, eight had tissue available for further analysis. Three additional specimens were obtained from Guangzhou, China, for a total of 11 specimens. All 11 specimens were positive for the presence of EBV. Of the seven with sufficient tissue to test for viral clonality, six were found to have clonal EBV DNA in the lesion.

A prospective serologic study conducted in Taiwan examined the role of EBV antibodies as predictive of subsequent NPC (Chien et al., 2001). The subjects were men residing in areas with the highest rates of NPC. A total of 9688 men were enrolled who had no evidence of NPC and who provided blood specimens. All specimens were tested for anti-VCA-IgA and anti-EBV DNase antibodies. The subjects were followed for up to 16 years, and 22 incident cases of NPC were identified, who were diagnosed after 1 year from enrollment. The prevalence at baseline of anti-EBV DNase was 12% and that for anti-VCA-IgA was 1.2%. The relative risk (RR) for developing NPC for persons with both markers at baseline, compared to those with neither, was 20.7 (95% confidence interval, 2.6–162). Other major risk factors for NPC include genetic susceptibility and related environmental exposures, especially dietary factors (IARC, 1997).

**Hodgkin Lymphoma**

Hodgkin lymphoma (HL) is a relatively common malignancy of young adults in Westernized populations, where the age-incidence curve is generally bimodal (Fig. 26–1) (see Chapter 45 for a detailed review). Since EBV was first identified, it was considered a candidate virus for this lymphoma. The hypothesis that age at infection with EBV may play an important role in the development of HL was suggested by the epidemiological similarities between HL among young adults and IM in economically advantaged populations. For both diseases, risk has been associated with higher social class and small family size (Gutensohn and Cole, 1977, 1981). These similarities suggest that exposure to the causative agent is delayed until young adult life. It has been almost always found that people with a history of IM have about a threefold increase in their risk of developing HL. In addition, a larger proportion of HL cases have elevated antibody titers to several EBV antigens, primarily VCA-IgG and EA-D, as compared to controls. Generally, some 30% to 40% of HL cases are found to have elevated titers. This has been a consistent finding in many studies over different geographic areas (Mueller, 1987).

A pilot study based on two cases of HL occurring prospectively among 25,802 persons with banked serum samples showed that in comparison to matched-controls, EBV antibodies were significantly elevated prior to diagnosis, in contrast to three other herpesviruses whose antibody titers were not elevated (Evans and Comstock, 1981). This study was then extended to include sera for more than 240,000 persons from five serum banks. In this extended study, 43 persons who developed HL between 1 and 13 years after blood collection were identified and matched with 96 controls (Mueller et al., 1989). EBV antibody analysis of these sera confirmed the findings of the pilot study, in that the proportion of patients with elevated antibody against VCA-IgG, IgA, and EBNA complex was significantly higher than that of the controls; this was associated with a three- to sevenfold increased risk of HL. Of interest, the cases had significantly decreased levels of IgM against the VCA, compared to controls (Mueller et al., 1989). More than half (56%) of the sera from pre-HL cases had elevated titers of one or more EBV antibodies, compared to 35% of controls. This pattern of EBV antibodies was quite different from that seen in prediagnosis serum samples from non-Hodgkin lymphoma patients, obtained from the same serum banks (Mueller et al., 1991); no significant differences were found for antibody titers against CMV.

A major breakthrough linking the EBV to HL was achieved by the application of molecular hybridization assays, as first reported by Weiss et al. (1987); these studies are summarized in detail elsewhere (Mueller, 1996). The studies consistently find that between 25% and 50% of HL cases are EBV-positive, as defined by the presence of EBV genome (which has been shown to be monoclonal) or of viral gene products. EBV positivity is more strongly associated with the histologic subtypes that connote more advanced disease, is higher in HL in children, and is somewhat lower among young adults (Glaser et al., 1997). As first reported by Pallesen et al. (1991), it has been further demonstrated that the EBV-positive tumor cells express a restricted latent infection phenotype of only EBNA-1+LMP1+LMP2A+ (Niedobitek et al., 1997; Ambinder and Weiss, 1999) (Table 26–2).

In parallel we have found that the typical HL EBV antibody pattern segregates with tumor EBV-positivity, while that for EBV-negative HL appears to be normal in both a case-control study (Chang et al., 2004) and in prospectively obtained blood specimens (L. Levin et al., personal communication).

Finally, the link to IM history was validated by Hjalgrim et al. (2003). In the follow-up of 38,000 Scandinavians with IM, the authors were able to characterize EBV status on biopsies from 29 of 40 cases of HL diagnosed ≥2 years after the diagnosis of IM. The RR of EBV-positive HL in this group associated with prior IM was 2.8 (1.7–4.6) and 1.1 (0.7–2.0) for EBV-negative HL.

**Biological Causal Mechanisms**

The EBV has evolved a number of inherently oncogenic mechanisms that ensure its survival (Young and Murray, 2003; Dolcetti and Masucci, 2003). These include its ability to transform resting B-cells into permanently growing lymphoblastoid cell lines in vitro. The LMP-1 protein—which is expressed in both a lymphoid tumor, HL, and an epithelial tumor, NPC—is in itself an efficient oncogene. LMP-1 plays a central role in transformation and in the up-regulation of a range of cellular factors, including the NFκB transcriptional pathway. What is actually astounding is that the great majority of humankind carry this virus through much of their lifetimes with no ill effects, being protected by the persistent surveillance of EBV-specific CTLs.
This protection is seen most clearly when normal carriers undergo therapeutic immune suppression for organ transplantation. In a small proportion of such cases, a spontaneous lymphoproliferation can occur, which can evolve to an EBV-positive mononuclear B-cell lymphoma. The infusion of EBV-specific CTL can reverse this malignant process (Rooney et al., 1995).

Cofactors
A consistent theme in the EBV-associated malignancies is the apparent effect of social environment on age at infection. The mechanism underlying these associations may likely relate to an age-related cytokine milieu. Other effects of social environment—such as that seen in the association of poverty and EBV-positive HL—may contribute. Genetic and dietary factors have been identified as likely of importance in NPC. Coinfection with HIV is a risk factor for both BL and for the EBV-positive HL. The increased risk among males that is seen for BL, NPC, and HL may reflect the protective effect of the relatively stronger type 1 immunity of women. Of potential interest, we have found in a recent case-control study on HL that aspirin, which selectively down-regulates the NFκB pathway, is apparently protective (Chang et al., 2004).

Prevention and Future Research
EBV is largely an unavoidable infection. The epidemiologic evidence suggests that EBV—like many viral infections—is encountered best in childhood. That would argue against overly protecting children from infection and in favor of preschool and day care experience for those children who have limited exposure to other children at home. Given the potential oncogenicity of EBV as well as the morbidity of IM, the development of an effective vaccine against EBV is warranted.

Potential interventions in early disease should be considered. For example, the therapeutic use of EBV-specific CTLs might be tested for early stage EBV-positive HL or NPC. The finding of an apparent protective effect of aspirin against HL needs to be confirmed.

Finally, EBV continues to be implicated in a surprising array of malignancies. Some of these connections—such as with breast cancer (Labrecque et al., 1995)—may not prove to be valid (Hermann and Niedobitek, 2003). Others, such as for gastric cancer (Niedobitek et al., 1992), appear to be valid (Takada, 1999), and the underlying biology and epidemiology should be pursued. In either case, the application of the principles and biomarkers that have guided the established models should aid in validating and defining new EBV-associated malignancy.

Hepatitis B Virus
Hepatitis B virus (HBV) is a DNA virus within the Hepadnaviridae family, which includes other mammalian and avian hepadnaviruses, such as the woodchuck hepatitis virus and the duck hepatitis B virus (Ganem, 1996). These viruses have a very narrow host range as well as a striking tropism for hepatocytes. Among the DNA viruses, hepadnaviruses are uniquely unusual in that they replicate via reverse transcription of a full-length RNA copy of the virus genome. The spherical HBV virion, which is called a “Dane” particle, is about 42 nm in diameter. The outer lipid, bilayer envelope contains the hepatitis B surface antigen (HBsAg). Within the inner nucleocapsid core (HBcAg) is a partially double-stranded, circular genome approximately 3.2 kb in size as well as a DNA polymerase.

The HBV genome contains four open reading frames for the S, C, pol, and X genes (Ganem, 1996; Lee, 1997). S encodes the envelope surface protein, HBsAg, and includes the two pre-S regions and the S region. Transcription beginning at the pre-S1 region produces the “large” S protein; at the pre-S2 region, the “middle” S protein; and at the S region, the “major” S protein. The C gene codes for the core protein, HBcAg, with the amino terminal region used for the synthesis of the HBV e antigen (HBeAg). The polymerase protein encoded by pol contains the DNA polymerase and reverse transcriptase functions. The role of X in the virus life cycle is less clear, although in vitro experiments have shown that it can transactivate transcription of viral as well as cellular genes.

Natural History of HBV Infection
Normally in acute HBV infection, HBsAg is usually the first marker detected, appearing within the first few weeks of infection (Hoofnagle and Schafer, 1986; Hollinger, 1996). HBeAg is present at about the same time as HBsAg and then is lost after a few days to a few weeks with subsequent seroconversion to antibody against HBeAg. Anti-HBcAG also appears during the early phase of infection, with the detection of IgM-specific antibody being a characteristic of acute infection. The HBe IgM antibody declines within about 6 months, but HBc IgG antibody generally persists indefinitely as a marker of past infection. Hepatitis symptoms tend to occur about 2 months post-infection, although the majority of acute infections are, in fact, subclinical. As HBV is cleared, antibody against HBsAg becomes detectable about 8 months after infection and subsequent to the disappearance of HBsAg.

In contrast to recovery from acute infection, with chronic HBV infection, HBsAg, along with antibodies to HBc, remains persistently detectable, and HBs antibody does not develop (Hoofnagle and Schafer, 1986; Hollinger, 1996). HBeAg can persist for years; normally, about 10–15% of HBV chronic carriers will lose their HBeAg per year and seroconvert to anti-HBe seropositivity (Alward et al., 1985; Evans et al., 1997). The loss of HBeAg appears to be associated with latency of infection, with no or little replication. HBsAg also can disappear over time and antibodies against HBs develop, but this occurs at a very low rate of about 1% annually. HBV can become integrated into the host genome over the course of chronic infection and likely represents an important step toward hepatocarcinogenesis (Thomas, 1990; DeFranchis et al., 1993; Ganem, 1996). The integrated virus is not usually replicatively competent, although HBsAg is synthesized and expressed on the surface of the infected hepatocyte.

The probability of chronic infection is closely and inversely related to the age at which infection with HBV occurs (Thomas, 1990; IARC, 1994a; Hollinger, 1996). Around 80% to 90%, or more, of infants born to infected mothers become chronic carriers (Beasley et al., 1977), whereas less than 10% of adolescents and adults will develop a persistent infection. Of children between the ages of 1 and 10, 20–40% will become chronic carriers, with the probability decreasing with increasing age over those years (McMahon et al., 1985). The situation is reversed with respect to the occurrence of clinically apparent hepatitis: about 30–50% of acutely infected adolescents and adults will experience symptomatic disease, compared to fewer than 5% of babies and young children. This difference in the course of initial infection is related to the competency of the host immune response to HBV. A strong immune response is required to clear the infection (Lee, 1997; Koziel, 1999). CTLs, primarily directed against HBV core antigens, kill infected hepatocytes. Cytokines, such as tumor necrosis factor-α (TNF-α) and IFN-γ, also appear to be important in inhibiting HBV replication in acute resolving infection. This inflammatory response results in immune-mediated damage of the liver, leading to acute hepatitis. Nonetheless, the damaged hepatocytes are eventually replaced, neutralizing antibody develops, and the liver returns to normal.

In chronic infection, the immune response is insufficient to completely eliminate the infection—as in the case of neutrdes who have immature type 1 immunity or in persons who are immunodeficient (Thomas, 1990; Hollinger, 1996). Thus, some infected hepatocytes persist and, in turn, infect other hepatocytes. The infected cells continue to be targets of the immune response, setting up cycles of hepatocellular injury and regeneration. Of note, infected young boys are slightly more likely to become chronic HBV carriers, despite an equal likelihood of infection at birth for both genders (London, 1981). About 70–90% of chronic HBV carriers will remain asymptomatic (Hoofnagle and Schafer, 1986). However, 10–30% will go on to develop chronic persistent or chronic active hepatitis. Of those carriers with chronic active hepatitis, approximately 1–2% per year will progress to cirrhosis. Both the hepatitis and cirrhosis can be clinically
silent. Hepatocellular carcinoma (HCC) develops at an annual rate of 1–6% among those with cirrhosis (Hollinger, 1996; Tabor, 1998). The latent period from infection to the occurrence of HCC is estimated to range from 30 to 50 years. About 15% to 25% of chronic HBV carriers will eventually die of some form of liver disease. Extrahepatic conditions related to HBV infection appear to be relatively rare and primarily include diseases associated with damage resulting from the formation of immune complexes (IARC, 1994a; Lee, 1997).

**Epidemiology of Infection**

HBV is primarily a blood-borne infection that is transmitted by percutaneous and percutaneous routes. The major modes of infection are via sexual contact, perinatal, and perinatal exposure. High levels of virus are found in blood and semen; more moderate viremia is evident in semen, vaginal fluid, and saliva. With respect to transmission by sexual contact, multiple partners and a history of sexually transmitted diseases appear to increase the risk of infection (Alter et al., 1989; IARC, 1994a; Hollinger, 1996). Sexual transmission of HBV is higher from men to women than the reverse. Parenteral exposure can occur via intravenous drug use as well as tattooing and body piercing. Medical personnel also are at risk of HBV transmission by contaminated needlestick or other injuries with sharp instruments. Before blood screening for hepatitis B core (HBc) antigen began, transmission with contaminated units was a source of infection; in addition, hemodialysis has been a route of HBV transmission.

Perinatal transmission occurs from the infected mother to her infant, usually by contact with maternal blood during delivery (Beasley, 1988). Some infected babies may acquire their infection in utero (Stevens et al., 1985; Li et al., 1986). The probability of perinatal infection is related to whether the carrier mother is also HBcAg-positive; the chance of infection is 70–90% for HBeAg-positive mothers versus 5–10% for HBeAg-negative, HBV-infected mothers (Beasley et al., 1977; Hollinger, 1996). Horizontal transmission of HBV also occurs in childhood. The actual modes of infection during this age period are not clear and likely multiple. In developing countries, the use of nondisposable, contaminated needles and syringes may play a role in transmission, particularly in the past (Ko et al., 1991). Transmission via skin lesions, human bites, and arthropods has also been suggested (Vall Mayans et al., 1990; IARC, 1994a). A study in Ghana found sharing of bath towels, of chewing gum or partially eaten candies, and of dental cleaning materials with an HBV carrier to be significant, independent risk factors for HBV infection in children aged 1 to 16 years (Martinson et al., 1998). Of interest, biting one’s fingertips in conjunction with scratching a carrier’s back also was associated with HBV seropositivity. The authors concluded that contact with objects contaminated with infectious saliva and/or blood represent important routes of HBV transmission in this age group. Childhood infection does appear to be associated with multiple infected siblings in the household (Whittle et al., 1991), also supporting possible contact-related transmission.

The WHO estimated that there were 400 million HBV carriers in the world in the year 2000 (Lee, 1997). The prevalence of chronic infection is high (28% HBsAg positivity) in China, Southeast Asia, and sub-Saharan Africa as well as in parts of South America and in Alaska (IARC, 1994a). Moderate levels of endemicity (2–7%) are found in eastern and southern Europe, the Middle East, Japan, and southern and northern Asia. The lowest prevalence of infection (<2%) occurs in the Americas, including the United States, western and northern Europe, and Australia.

The global distribution of chronic HBV infection is a function of the relative contribution of the different routes of infection on the level of endemicity. Perinatal infection is an important transmission route in high-endemic areas, particularly in China and other parts of South Asia where up to half of the chronic infections are due to neonatal transmission (Beasley, 1988; IARC, 1994a). The higher frequency of perinatal infection in these countries, than in other endemic areas, appears to be related to the proportion of carrier mothers who are positive for HBeAg (Beasley et al., 1977). For reasons that are not known, the prevalence of HBeAg positivity is higher among infected Asian women than among infected women in other endemic areas, such as Africa (IARC, 1994a). Childhood infection also plays an important role in the transmission of HBV in highly endemic regions as well as in areas with more moderate levels of chronic infection. In low-endemic areas, transmission occurs mainly during adulthood and adolescence, with sexual contact and parenteral exposure as the predominant modes of HBV infection. Although HBV infection of adults and adolescents takes place in areas with moderate or high levels of endemicity, its contribution to the prevalence of chronic carriers is relatively minor, as most of such infections would be cleared.

In the United States, 0.33% of the population has been estimated to be HBV chronic carriers, based on data from NHANES III, conducted from 1988 to 1994 (McQuillan et al., 1999). The sample prevalence of infection was similar to that found in NHANES II (1976 to 1980). The proportion of persons ever-infected with HBV differed little between the two surveys: NHANES III, 4.9%, age-adjusted: NHANES II, 5.5%. Men were more likely to have been infected than were women (5.7% vs. 4.1%, respectively, NHANES III). The age-specific prevalence of infection in the United States appears to be quite low, until about the teenage years when it begins to increase, emphasizing the importance of sexual transmission and high-risk parenteral behaviors as routes of infection (Goldstein et al., 2002). Non-Hispanic blacks (12.8%) had higher levels of ever infection than did non-Hispanic whites (2.8%) and Mexican Americans (4.8%).

**HBV Biomarkers**

Several HBV antigens and antibodies to viral antigens can be routinely detected in serum, using commercially available assays (IARC, 1994a; Hollinger, 1996). As shown in Table 26–3, the chronic carrier state of infection is marked by the presence of HBsAg for more than 6 months. Antibody to HBsAg represents the neutralizing antibody and is indicative of recovery from infection and immunity to reinfection—the HBV vaccine induces the production of anti-HBs. HBeAg serves as a marker of high viral replication; in contrast, detection of antibody to HBe points to a low level of replication. Although HBeAg itself is not usually detectable in serum, both total antibody as well as IgM-specific antibody directed against HBeAg is used to distinguish past or chronic HBV infection from acute infection, respectively. Of note, assays for antibodies against HBeAg have been found to be not very specific, particularly at low titers; however, high titers, without the concurrent detection of HBsAg, can signify a low-level carrier. In addition, PCR methods can be used to detect the presence of HBV DNA in the serum.

**Hepatocellular Carcinoma**

 Chronic HBV infection has been established as a major cause of HCC (IARC, 1994a). The global incidence of HCC is strongly correlated with the prevalence of chronic HBV infection (Maupas and Melnick, 1981), with high rates of liver cancer found in Southeast Asia and sub-Saharan Africa, and low rates in the United States and Europe (Muñoz and Bosch, 1987). Researchers at IARC estimated that 52% of all liver cancer cases worldwide in 1990, about 229,000, could be attributed to chronic infection with HBV (Parkin et al., 1999). Most of the burden of HBV-associated liver cancer can be found in developing countries (attributable fraction, 59%), primarily Melanesia/Micronesia (76%), China (70%), and Africa (66%). In contrast, less than a quarter of liver cancer cases in developed countries are attributable to HBV; in North America and Australia, the estimate is 9%.

A substantial body of epidemiologic evidence supports the etiologic role for chronic HBV infection in hepatocarcinogenesis (Muñoz and Bosch, 1987). Overwhelmingly consistent findings have been accumulated over two decades of research studies conducted in more than 25 different countries (IARC, 1994a). In the nearly 100 or more case-control studies evaluating this association, RR estimates have generally ranged from about 3 to 30, with a few reporting stronger associations (IARC, 1994a; Donato et al., 1998; Kuper et al., 2000a). Even more compelling evidence has been provided by more than 15 cohort studies that have followed HBV carriers over time (IARC...
1994a; Mori et al., 2000; Evans et al., 2002). In these studies, RR from 5 to more than 100 were observed. Probably the most well-known cohort study is that conducted in Taiwan by Beasley et al. (1981), which involved more than 19,000 male government employees followed for about 10 years and reported an RR of 103. The RR estimates tend to be higher in the cohort studies than in the case-controls because serum HBsAg (as biomarker of chronic infection) may disappear in some cases during the development of HCC (Huo et al., 1998).

HBV DNA has been detected in the tumor tissue of more than 90% of HCC cases with HBsAg in their serum as well as in 10–20% of cases without HBsAg (Bréchot et al., 1982; Popper, 1988; Tabor, 1998). These HBV sequences appear to be clonally integrated into the genome of the infected hepatocyte, with no common integration site for the virus identified. Multiple copies of the HBV genome are found that could lead to the generation of random chromosomal abnormalities, including allelic loss (Tabor, 1994; Bréchot et al., 2000). Once integrated into the host genome, HBV no longer appears able to replicate, most likely as a result of the frequent rearrangement and deletions occurring in the viral genes. Of note, altered sequences from the X and pre-S2/S genes are preferentially retained.

**Mechanisms of Carcinogenesis**

The exact mechanisms by which HBV induces hepatocarcinogenesis are essentially unknown (London and Buetow, 1988). HBV does not appear to be directly cytopathogenic. As discussed above, HBV-associated liver injury arises from the cell-mediated immune response to the virus and from bystander damage related to the release of cytokines. As much as 80% of HCC is observed to occur in conjunction with cirrhosis (Okuda et al., 1982; Hollinger, 1996; Tabor, 1998). Thus, chronic HBV infection could lead to HCC through continuous cycles of hepatocyte necrosis and regeneration that would "promote" acquired mutations that exist in the liver cells. Hepatocytes, in which HBV has become integrated, no longer express HBcAg and would not be targets for immune clearance, leading to their selective regeneration. Because it has the capability both to initiate, by virtue of its ability to integrate into the host genome, and potentially cause mutation as well as to promote hepatocarcinogenesis via constant immune-mediated inflammation and cell turnover, HBV has been viewed as a "complete carcinogen" (Trichopoulos et al., 1987; Tabor, 1994).

The woodchuck hepatitis virus appears to induce liver cancer via insertionmutagenesis at the myc gene location (Pineau and Tiollais, 1997). Although the evidence does not suggest that HBV acts in a similar manner, in vitro experiments have shown that proteins encoded by the X and pre-S2/S regions of the HBV genome, are able to transactivate several cellular oncogenes, including c-myc and c-fos (Kekulé et al., 1990; Balsano et al., 1991; Tabor, 1994) which are overexpressed in HCC tumor cells (Koshy and Wells, 1991). These alterations may be important for transactivation. The native pre-S2/S middle protein does not appear to have transactivating properties, and the truncated X protein is a stronger transactivator than is the wild-type version. In addition, the X protein has been shown to bind the p53 tumor suppressor protein and to inhibit its function in HCC tissue as well as in transgenic mice (Tabor, 1997; Ueda et al., 1997). HCC has been reported to develop at a very high frequency in mice transgenic for the X gene, particularly in male mice (Kim et al., 1991; Ueda et al., 1997). In addition, in vitro studies indicate a possible role of the HBV X protein in cell cycle control and apoptosis (Bréchot et al., 2000).

With respect to growth factors, the detection of transforming growth factor-α (TGF-α) is closely linked to that of HBsAg in HCC tissue as well as in nontumorous tissue (Tabor, 1994). It is possible that HBV-related liver regeneration leads to increased levels of TGF-α, and/or that it acts in conjunction with HBV in the process of hepatocarcinogenesis. Last, some data exist that suggest that insulin-like growth factor-II is overexpressed in HBV-associated HCC (Lee et al., 1998).

As previously mentioned, clonally integrated HBV DNA is found in the liver tissue from HCC cases who are negative for HBsAg. Indeed, low levels of HBV DNA can be detected in HCC cases as well as in healthy blood donors who are HBsAg-negative (Huo et al., 1998; Bréchot et al., 2000; Yotsuyanagi et al., 2001; Tabor, 2002). Frequently in such individuals, the only other marker of HBV infection found is anti-HBe. Such "silent" or "occult" HBV infections are believed to be etiologically relevant in the development of HCC, perhaps with other cofactors acting as promoters of hepatocarcinogenesis. The role of occult HBV coinfection may be particularly relevant with respect to the pathogenesis of HCV (Cacciola et al., 1999; Marusawa et al., 1999b; Tabor et al., 2002).

**Cofactors**

The establishment of chronic HBV infection at a young age in childhood appears to increase the risk of HCC occurrence, based on data from case-control studies (Muñoz et al., 1989; Hsieh et al., 1992; Kuper et al., 2000c). In addition, HCC is much more frequent in persons younger than 40 in populations with a high incidence of liver cancer than in populations in which the risk of liver cancer is relatively low (Bosch, 1997). Male carriers are at a higher risk of HCC as well as of cirrhosis than are female carriers (Beasley, 1988; IARC, 1994a; Evans et al., 2002); this male predominance is more apparent in developing countries where HCC incidence and HBV endemicity are high. Although some of the gender difference may be due to higher prevalence of other cofactors in men (e.g., high alcohol consumption), endogenous hormones also may play a role. A cohort study in Taiwan found a significant fourfold increase rate of HCC for men in the highest tertile of testosterone level (Yu and Chen, 1993). In contrast, a cohort study in Shanghai reported a modest nonsignificant 50%
increased risk among HBV carriers with the highest tertile of testosterone (Yuan et al., 1995). In an additional study by the researchers in Taiwan, the relationship between lower CAG repeats in the androgen receptor (AR) gene, which has been associated with prostate cancer in men and the incidence of HCC in male HBV carriers, was investigated (Yu et al., 2000). A twofold increased risk for HBV carriers with 20 or less CAG repeats in AR was observed; a possible synergistic effect of high testosterone levels also was suggested.

Aflatoxins, which are produced by molds that contaminate food staples stored in hot, humid climates, have been determined to be a human carcinogen (IARC, 1993). They are hypothesized to be an important risk factor for HCC in some areas of the developing world (Montesano et al., 1997). In nested case-control studies of HCC among men in Shanghai (Qian et al., 1994) and Taiwan (Wang et al., 1996), a strong positive interaction was found between detectable urinary aflatoxins and HBsAg seropositivity. The particular p53 mutation, believed to be associated with aflatoxin exposure (the 249th mutation), occurs at a higher frequency in HBV-associated HCC. Experimental evidence from studies in woodchucks and HBV-transgenic mice further support a synergistic effect of HBV chronic infection and aflatoxin consumption on the incidence of HCC (Montesano et al., 1997). A meta-analysis of HCC cases estimated a significant interaction between HBV positivity and a high level of aflatoxin exposure (Stern et al., 2001). However, HBV infection did not appear to modify the effect of high aflatoxin exposure on the proportion of HCCs with the 249th mutation.

It is known that the consumption of alcohol is hepatotoxic, although not necessarily mutagenic. Moreover, the epidemiologic data indicate that excessive drinking and alcoholic cirrhosis increase the risk of HCC (Kuper et al., 2000b; Donato et al., 2002). Several studies suggest that more than an additive effect of heavy alcohol consumption and chronic HBV infection exists with respect to the development of HCC, although the findings have been somewhat inconsistent (Bréchet et al., 1996; Chen et al., 1997; Yu et al., 1997; Kuper et al., 2000b; Mori et al., 2000; Donato et al., 2002). The proposed mechanism of action is that alcohol drinking further exacerbates HBV-induced hepatocellular damage.

A role for smoking in hepatocarcinogenesis is biologically plausible (Kuper et al., 2000b). Whereas some studies have found a positive, although not necessarily significant, interaction between increased smoking and HBV infection for HCC (Chen et al., 1997; Mori et al., 2000), others have not (Tanaka et al., 1992; Kuper et al., 2000b; Evans et al., 2002).

The evidence regarding an effect of HIV coinfection on the progression and pathogenesis of HBV has not been clear. Some studies have reported lower HBV levels as well as lower liver enzyme values in persons coinfected with HIV, implying a weaker immune response to HBV (Gilson et al., 1997; Colín et al., 1999). However, in a few studies, more liver disease has been observed in HBV carriers with HIV than in those without HIV (Colín et al., 1999; Thio et al., 2002), although not in others (Lee, 1997). A strong immune response during acute infection clearly is important with respect to clearance of HBV (Lee, 1997; Koziel, 1999). At the same time, among persons with chronic, persistent infection, progression to liver disease most likely is a consequence of HBV-specific immune-mediated damage. Thus, it seems plausible that host immune function, and factors affecting the balance between immune control of HBV infection and immune-induced liver injury, should be important in the natural history of the infection.

### Prevention and Future Research

The best prevention of HBV-associated liver cancer is through the elimination of chronic HBV infection. The development of a safe and effective vaccine against HBV infection makes this goal attainable. HBV mass vaccination programs were begun in the early to mid-1980s in HBV-endemic populations, including in Africa, China, Taiwan, and the United States (Anand and Hollinger, 1997; Blumberg, 1997). Because the development of chronic HBV infection is much more likely in younger children, who also have the highest risk for HCC, immunization during infancy or early childhood is the most effective strategy for reducing the burden of HBV. Accordingly, the childhood vaccination programs initiated in HBV-endemic areas have led to marked reductions in the prevalence of carriers in those populations (Chen et al., 1996; Blumberg, 1997). A most striking finding has been the observation of a significant decline in the incidence of childhood HCC in Taiwan between 1981 and 1994, subsequent to the introduction of the nationwide immunization effort against HBV (Chang et al., 1997). In the United States, the HBV vaccine has routinely been administered to infants since the early 1990s (Goldstein et al., 2002). As of 2002, programs targeted at universal immunization of infants existed in more than 100 countries worldwide (WHO, 2002).

Interferon-α therapy is used to treat chronic hepatitis due to HBV infection. Wong and colleagues (1993) performed a meta-analysis of the published results from 15 clinical trials that evaluated the effect of IFN-α treatment of HBeAg-positive patients with chronic hepatitis B. About one-third showed a loss of HBeAg and/or HBV DNA. More recent studies have confirmed the 30–50% clearance of HBeAg or HBV DNA after IFN-α therapy for chronic hepatitis B, with lower rates of disease progression suggested for those who responded to treatment than for those who did not (Niederer et al., 1996). The effect of IFN on the development of HCC has been examined in a few studies of patients with HBV-related cirrhosis (Tabor, 2003). The studies, which were conducted in Singapore (Oon, 1992), Italy (Mazzella et al., 1996), and Japan (Ikeda et al., 1998), each reported fairly substantial reductions in the occurrence of HCC in the treated cirrhotics compared to the untreated cirrhotics, despite differences in study design with respect to the treatment regimen and the length of follow-up. In the study in Italy, the HCC cases that occurred in the treated patients were only among those who did not respond to the IFN therapy.

Thus, decreased disease progression, including the development of HCC among persons with HBV-induced liver injury, appears possible with IFN treatment. However, the side effects related to such therapy can be problematic. The development of more effective, less toxic treatments would be important in minimizing morbidity and mortality for those with chronic HBV infection. In parallel, the identification of those subgroups of HBV carriers (as defined by age, gender, and biomarkers, for example) who can benefit from therapeutic interventions should be determined.

### HEPATITIS C VIRUS

Hepatitis C virus (HCV) is a small, enveloped RNA virus. HCV has been classified as a separate genus in the Flaviviridae family, which includes such human pathogens as the yellow fever and dengue viruses as well as the hepatitis G virus (Lauer and Walker, 2001). The virus was first identified in 1988 by Choo, Kuo, and colleagues as the biologic agent likely responsible for most non-A, non-B hepatitis in the United States (Choo et al., 1989; Kuo et al., 1989). Using state-of-the-art molecular techniques available at the time, the investigators screened an enormous number of clones prepared from the plasma of an experimentally infected chimpanzee. They eventually obtained a single cDNA clone that allowed them to isolate and sequence the virus and lent molecules to the field that have been used to classify the virus as a separate genus in the Flaviviridae family. The positive-sense, single-stranded viral genome, which is approximately 9.6 kb in length, codes for the core nucleocapsid protein, two envelope (E) glycoproteins (E1, E2), and six nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B) (De Francesco, 1999). Neutralizing antibodies appear to be directed against the E2 protein (Koziel, 1997; Ferrari et al., 1999). Within the E2 coding sequence, there are two hypervariable regions; these regions are important with respect to the virus’s ability to evade neutralization by the immune system, by leading to the generation of multiple quasi-species of genetically related HCV variants within the infected host, similar to HIV (Pawlotsky, 2002). With respect to the nonstructural proteins, NS2, NS3, and NS4A have protease functions, with NS3 also demonstrating RNA helicase activity; NS5B functions as a RNA-dependent RNA polymerase and is important in genome replication, perhaps through the synthesis of a negative-strand intermediate (De Francesco, 1999). Lacking reverse transcriptase, HCV does not integrate into the host genome (Choo et al., 1989).
HCV infects hepatocytes and also may be lymphotropic (Wang et al., 1992; Bartolome et al., 1993). In vivo, the virus appears to replicate at a very high rate, producing as many as 10^{12} virus particles per day (Neumann et al., 1998). Until recently, HCV had not been grown successfully in cell culture, limiting the study of its replication and the viral life cycle. However, the generation of functional subgenomic replicons in a human hepatoma cell line (Lohmann et al., 1999) represented an important step toward the eventual development of in vitro systems that can produce infectious virus particles (Wakita et al., 2005; Zhong et al., 2005; Lindenbach et al., 2005). At present, the chimpanzee is the only experimental animal that has been infected with HCV. No small animal model currently exists for studying the infection, although chimeric mice with human hepatocyte engraftments have been created and can sustain HCV infection of the xenogeneic cells (Mercer et al., 2001; Meuleman et al., 2005).

Natural History of HCV Infection

In acute infection with HCV, virus RNA is detectable within 2 weeks after exposure (Hoofnagle, 2002). A few weeks later, serum alanine aminotransferase (ALT) levels, indicating liver dysfunction, begin to rise, with symptoms occurring soon after. However, less than 30% of persons newly infected with HCV will present with clinically apparent hepatitis. After the acute infection, periodic elevations in ALT can be observed and often parallel changes in HCV RNA load (Alter et al., 1992; Hoofnagle, 2002; Okayama et al., 2002). Antibodies against HCV become detectable about 8–12 weeks after exposure. The presence of HCV antibodies appears to be life-long; however, in some persons who have cleared their HCV infection, these antibodies may eventually disappear (Alter et al., 1992; Wiese et al., 2000; Hoofnagle, 2002).

A vigorous CTL response is thought to be important for clearance of infection (Lechmann et al., 1996; Spengler et al., 1996; Koziel, 1997; Takaki et al., 2000). A strong T-lymphocyte response and low or undetectable antibody levels have been found in acute hepatitis patients who clear their HCV infection, whereas a weak T-cell proliferative response and high antibody titers appear to characterize those patients who develop chronic hepatitis (Spengler et al., 1996).

Some individuals are able to clear HCV infection, as marked by the loss of detectable RNA. However, the majority of newly acquired infections, as much as 75–85%, appear to become chronic as demonstrated by the persistence of HCV viremia for more than 6 months. Population-based, cross-sectional studies, of the prevalence of HCV RNA positivity among anti-HCV seropositives, have found similar proportions of persistence (Alter et al., 1997, 1999; Bellentani et al., 1999; Arduino et al., 2001; Hyams et al., 2001). However, the development of chronic infection has been reported to be lower, around 55% in some populations (Rodger et al., 2000; Hoofnagle, 2002; Seeff, 2002), including among young mothers infected with HCV by contaminated anti-D globulin (Kenny-Walsh et al., 1999; Wiese et al., 2000). In general, persistence of infection may be less frequent in women, younger persons, and individuals with symptomatic acute infection (Alter et al., 1999; Bellentani et al., 1999; Kenny-Walsh et al., 1999; Inoue et al., 2000; Wiese et al., 2000; Hoofnagle, 2002) and more frequent in African Americans and persons with immunodeficiency (Thomas DL et al., 2000; Seeff et al., 2001; Hoofnagle, 2002).

Epidemiology of Infection

In terms of transmission, parenteral exposure to contaminated blood appears to be a particularly efficient route of HCV infection. Prior to the screening of the blood supply for anti-HCV, which began in 1990 in the United States, transfusion of contaminated blood and blood products was an important source of transmission (Alter et al., 2000). As a result, the prevalence of anti-HCV is extremely high among persons with hemophilia, ranging from 50% to 95% (Rall and...
Dienstag, 1995). However, injecting drug use represents the most frequently reported risk factor for HCV infection in the United States and other Western populations, even among blood donors whose admitted intravenous drug use was often in the distant past (Conry-Cantilena et al., 1996; Alter et al., 1999, 2000; Murphy et al., 2000). Among active injecting drug users, the level of infection is as high as observed for those with hemophilia. HCV appears to be acquired relatively quickly after intravenous drug use is begun and to be transmitted more readily than HIV. In some studies, intranasal cocaine use also was associated with anti-HCV seropositivity, independent of history of intravenous drug use (Conry-Cantilena et al., 1996), although other studies have not observed this association (Murphy et al., 2000).

Other percutaneous exposures associated with the transmission of HCV include hemodialysis, organ transplantation, and accidental needlesticks in the health care setting (Rall and Dienstag, 1995; Alter et al., 2000). Data from Japan have implicated the use of nondisposable needles, syringes, and other medical instruments, after the Second World War and prior to the 1970s and 1980s, as a possible important route of HCV transmission in that country (Hayashi et al., 1995). Several folk remedies practiced in Japan that involve possible percutaneous exposure, such as acupuncture, also have been identified as potential sources of HCV infection (Kiroyawa et al., 1994; Noguchi et al., 1991). Studies in Italy and Taiwan also have reported an association between exposure to nondisposable needles and syringes and infection with HCV (Chen et al., 1995; Chiaromonte et al., 1996). In Japan, parenteral antischistosomal therapy campaigns likely are responsible for the very high prevalence of HCV in that country; transmission likely resulted from insufficiently sterilized syringes during the mass intervention efforts (Frank et al., 2000).

In addition, tattooing, body piercing, and sharing of razors have been reported as possible routes of HCV transmission, although the data have been rather limited and largely inconclusive (Conry-Cantilena et al., 1996; Alter et al., 2000; Murphy et al., 2000).

Nonpercutaneous transmission of HCV also occurs, albeit at a relatively low rate. This route of infection appears to be rather inefficient, particularly in comparison to other blood-borne infections such as HBV and HIV, or even HTLV-I. Although HCV likely is transmissible via sexual contact, the data concerning the relative importance of sexual transmission in HCV infection are somewhat conflicting. An increased prevalence of HCV has been reported for sexually transmitted disease clinic populations and for persons with multiple sexual partners, including prostitutes (Rall and Dienstag, 1995; Alter et al., 1999, 2000; Terrault, 2002). However, such groups may engage in other high-risk behaviors, which could have confounded some of the reported findings. In contrast, almost no transmission has been observed in the long-term, monogamous sexual partners or spouses of HCV-infected hemophiliacs (Terrault, 2002) and recipients of contaminated anti-D immunoglobulin (Meisel et al., 1995). Although some studies from Japan have reported evidence of sexual transmission of HCV within marriage (Akahane et al., 1994; Chayama et al., 1995), our cohort study as well as other studies have not supported this route of infection as having a major impact on the spread of HCV in Japan (Nakashima et al., 1995; Tanaka et al., 1997; Okayama et al., 2002). A transmission rate of <1% per year has been estimated for persons in monogamous relationships; the frequency is somewhat higher if an individual has multiple sex partners or is HIV-infected (Terrault, 2002).

Transmission from HCV-infected mothers to their babies has been reported in studies conducted in the United States, Japan, and Europe, although the probability is rather low, at 4–7% (Ohito et al., 1994; Zanetti et al., 1995; Roberts and Yeung, 2002). Perinatal transmission is hypothesized to take place in utero and/or at the time of delivery and not via breastfeeding. High virus titer as well as HIV coinfection in the mother appears to be important for transmission to occur.

The seroprevalence of HCV infection ranges from about 0.1% to 5% in most populations worldwide (Lavanchy and McMahon, 2000). The WHO estimates that approximately 3% of the world’s population is infected with HCV. The overwhelming majority of countries with available data have a seroprevalence of less than 2.5%. More intermediate prevalences, up to 10%, are found in Brazil, parts of Africa, and China. The highest reported level of infection is in Egypt, with estimates of 20–30% or more. Pockets of high seroprevalence also occur within areas of relatively low endemicity, as has been observed in some areas of Japan, where the frequency of anti-HCV positivity can be greater than 10% (Kiroyawa et al., 1994; Nakashima et al., 1995; Tanaka et al., 1997). In the United States, data from NHANES III estimate the prevalence of anti-HCV seropositivity to be 1.8% in the general population (Alter et al., 1999). The prevalence of infection is higher among non-Hispanic blacks and Mexican Americans than among whites. A slightly higher prevalence of anti-HCV among men also has been reported in the United States (Murphy et al., 1996; Alter et al., 1999). With respect to age, there appears to be a peak in prevalence among 30- and 40-year-olds. An explanation for the unimodal age distribution of HCV seroprevalence in the United States is that it reflects HCV infection via intravenous drug use during the late 1960s through the early 1980s (Murphy et al., 1996; Alter et al., 2000).

In populations with more endemic levels of infection, such as in Japan, we and others have found a somewhat different age- and sex-specific pattern of HCV seroprevalence (Hayashi et al., 1995; Nakashima et al., 1995; Okayama et al., 2002). There, the seroprevalence of anti-HCV tends to increase through the fifth decade and then is fairly stable. Also, men and women have about the same frequency of infection. This distribution of infection likely reflects differences in the timing and mode of infection between Japan and the United States.

HCV Biomarkers

The HCV RNA genome can be detected by reverse transcriptase PCR (RT-PCR) methods, with detection limits of the commercial assays as low as 50–100 IU/mL (Pawlotsky, 2002). Quantification of HCV RNA level can be obtained by RT-PCR or branched DNA signal amplification, although these assays are less sensitive than the qualitative assays to detect HCV RNA. Real-time PCR, based on the TaqMan system, may offer a more sensitive alternative for the quantification of HCV viral load (Takeuchi et al., 1999). In addition, enzyme immunoassays (EIAs) exist that can measure the amount of HCV core antigen (Aoyagi et al., 1999; Komatsu and Takahashi, 1999; Widell et al., 2002). Detection of core antigen appears to correlate very well with HCV RNA (Komatsu and Takahashi, 1999; Pawlotsky, 2002). Although the first versions of these assays could not detect HCV core antigen below 20 KIU/mL of HCV RNA (Bouvier-Alias et al., 2002), newer assays are more sensitive (Aoyagi et al., 1999; Tanaka et al., 2000) and may prove to be a more cost-effective, simpler means of detecting and quantifying HCV viremia on a population level.

Antibodies to HCV are most frequently measured using EIAs (Pawlotsky, 2002). Second-generation versions of these assays employ recombinant proteins derived from the core, NS3, and NS4 regions; the third-generation EIAs also include antigens from the NS5 region. Immunoblot testing can be used to confirm anti-HCV seropositivity, which was more important for earlier, less specific versions of the EIAs, particularly in populations with a low prevalence of infection, such as blood donors.

Six major genotypes of HCV, each with a number of subtypes, have been identified throughout the world (Simmonds et al., 1993). The six primary genotypes differ genetically from one another by approximately 30%; within a given genotype, the genetic variation is about 20% (Simmonds, 2000). The relative frequency of the HCV genotypes and subtypes does vary from country to country and even between different populations of the same country.

Hepatocellular Carcinoma

IARC classified HCV as a human carcinogen in 1993, primarily on the strength of epidemiologic data (IARC, 1994a). Parkin et al. (1999) have estimated that 25% of liver cancer cases occurring worldwide in 1990 could be attributable to HCV infection. The greatest burden excluding liver cancer caused by HCV is in Africa, Japan, and Oceania.
Mechanisms of Carcinogenesis

The mechanisms underlying the hepatocarcinogenic effect of HCV are not well understood. As a non-integrating virus, HCV is not likely to act as a cancer initiator in the usual sense. Because up to 90% of HCV-associated HCC arises within a cirrhotic liver (Alier and Seeff, 2000), HCV infection may lead to cancer through a promoting process of recurring cycles of cell death and regeneration, as would occur with cirrhosis (Tabor, 1998). It has been suggested that the presence of HCV-specific CTLs in liver tissue indicates T-cell-mediated toxicity with concomitant hepatocyte lysis (Koziel, 1997; Ferrari et al., 1999). HCV-associated liver cancer occurs in somewhat older patients and in conjunction with more severe disease than does HBV-associated HCC (Shiratori et al., 1995). Thus, an extended duration of liver cell damage—progressing from chronic hepatitis to cirrhosis to HCC—may be critical in hepatocarcinogenesis, resulting in an accumulation of mutations over an extended period in a stochastic manner (Kiyosawa et al., 1990; Tabor, 1998) (Fig. 26-5). It should be noted, however, that HCV-associated HCC has been reported in the absence of cirrhosis (Alberti et al., 1992; De Mitri et al., 1995).

The HCV core protein may be able to interfere with cell-cycle control and thus inhibit apoptosis (Ray et al., 1996b; Chung and Liang, 1999). In particular, the core protein may affect apoptosis induced by TNF-α, which is important in the immune response to viral infection (Marusawa et al., 1999a). In addition, there have been reports that the core protein can repress transcription from the promoters of the genes for the tumor suppressors p53 and retinoblastoma protein (Kim et al., 1994; Ray et al., 1997). Moreover, the N-terminal half of the NS3 protein of HCV as well as the viral core protein have been shown to transform cells in vitro (Sakamura et al., 1995; Ray et al., 1996a). Of further interest is the observation that mice transgenic for the HCV core gene develop HCC (Moriya et al., 1998). Experimental data also suggest that HCV replication may increase the expression of TGF-α and insulin-like growth factor-II, both of which are likely important in hepatocyte transformation (Tanaka et al., 1996).

Cofactors

A number of cofactors have been identified for HCV−induced HCC. Older age at infection and male gender appear to be associated with liver disease progression among HCV carriers (Poyaud et al., 1997; Freeman et al., 2001; Seeff, 2002). The interaction of alcohol drinking with chronic HCV infection in liver disease pathogenesis has been evaluated in a number of epidemiologic studies, with the evidence convincingly supporting that heavy alcohol consumption and HCV infection act synergistically to increase the progression of liver disease and the development of HCC (Shen et al., 1996; Poyaud et al., 1997; Tagger et al., 1999; Mori et al., 2000; Donato et al., 2002; Peters and Terrault, 2002). In cohort studies of HCC in Japan (Mori et al., 2000) and Taiwan (Sun et al., 2003), a positive interaction has been observed between cigarette smoking and HCV infection.

The hypothesis that HBV and HCV infections interact in the development of HCC has been supported by a number of reports (Donato et al., 1998; Tagger et al., 1999). In a meta-analysis of 21 case-control studies, Donato et al. (1998) estimated a greater than additive effect of the two viruses, with an OR of 165 for dual infection, 17.3 for infection with HCV alone, and 22.5 for infection with HBV alone. Of note, a negative correlation between the detection of anti-HCV and HBsAg within an individual has been observed, suggesting virus interference (Tanaka et al., 1991; Donato et al., 1998; Tagger et al., 1999; Thomas et al., 2000). Thus, when coinfection does occur, it may lead to enhanced hepatic pathogenesis and cancer development. Coinfection with HIV also appears to be associated with more rapidly progressing HCV-associated liver disease (Thomas, 2002). A meta-analysis of eight cohort studies found a significantly elevated rate of decompensated liver disease in persons coinfected with HIV and HCV, compared to those infected only with HCV (Graham et al., 2001). Of note, HCV RNA levels are reported to be higher in HIV-infected individuals (Eyster et al., 1994; Telfer et al., 1994; Thomas, 2002). In addition, we have observed a positive interaction between HCV and HTLV-I, with respect to liver cancer occurrence in the Miyazaki Cohort Study (MCS) (Boschi-Pinto et al., 2000). Thus, it seems plausible that immune suppression inhibits the effective control of HCV, with a resulting enhancement of liver damage, exacerbation of liver disease, and promotion of the hepatocarcinogenic process.

The host immune response to HCV infection likely is very important in the persistence of this infection and its induction of HCC. As with other chronic oncogenic infections controlled by CTLs, the antibody response may reflect persistent viral antigen expression and not a protective effect. Of interest, a study in Japan found an increased risk of HCC associated with very high antibody titers to HCV (Mori et al., 2000). In addition, several HLA genotypes have been found to
be related to both clearance of HCV (Barrett et al., 1999; Mangia et al., 1999) and liver disease progression (Aikawa et al., 1996; Kuzushita et al., 1998; Mangia et al., 1999). In particular, significant associations have been reported for alleles from the HLA class II DRB1 and DQB1 loci.

**Prevention and Future Research**

HCV is a serious public health problem in populations with high levels of infection. With the recent increases in HCC observed in the United States (El-Serag and Mason, 1999), there is growing concern that in a pattern similar to Japan (Okuda, 1997), this trend will continue over the next 20 years, primarily as a result of HCV infection (Armstrong et al., 2000).

In terms of prevention, no vaccine currently exists. The development of a vaccine has been impeded by substantial virus strain variation as well as the existence of multiple quasi-species within the infected host and the need for a strong cellular immune response to clear and control infection (Prince, 1994). Fortunately, interruption of parenteral transmission has led to marked decreases in the incidence of HCV infection. The screening of the blood supply for HCV antibodies has substantially reduced post-transfusion hepatitis C cases in Japan (Japanese Red Cross, 1991), Taiwan (Wang et al., 1995), and the United States (Alter, 1997; Tobler and Busch, 1997). In addition, the implementation of disposable needle and syringe usage and other changes in medical practices appears to have been effective in diminishing HCV transmission in Japan (Okuda, 1997) and Taiwan (Sung, 1997). Although the prevalence of HCV among intravenous drug users is shockingly high, increased efforts to prevent and treat drug use as well as to reduce sharing of injection paraphernalia (Thorpe et al., 2002) will be important in limiting new infections in this high-risk population.

Data from clinical trials suggest that treatment with IFN-α in combination with ribavirin results in sustained loss of HCV RNA in 40–50% of patients with chronic HCV, which is higher than the approximately 20% observed with IFN-α monotherapy (McHutchinson et al., 1998; Mangia et al., 2001). A number of studies have evaluated the direct effect of IFN-α treatment on the incidence of HCC (Tabor, 2003). Although issues have been raised concerning variations and problems in study design, the results of these studies suggest that IFN-α may reduce the occurrence of HCC in treated individuals, with a stronger effect being found for responders ( virologic or biochemical). Thus, chemotherapy may serve as a means of preventing the development of HCC in chronic HCV carriers. Continued improvements in treatments available (DiBisceglie and Hoofnagle, 2002) also would be beneficial in decreasing the burden of HCV-associated disease.

Future research should focus on clarification of the oncogenic mechanisms of HCV infection, with particular focus on the role of host immunity. The delineation of the natural history of infection can lead to the identification of high-risk individuals and, potentially, to the modification of risk. Such studies in community-based and special exposure cohorts should also provide more realistic estimates of disease outcomes for infected individuals. These research challenges will require multidisciplinary teams of epidemiologists, virologists, immunologists, and clinicians if progress is to be made quickly and cost-effectively.

**HUMAN HERPESVIRUS 8**

Human herpesvirus 8 (HHV8), or Kaposi sarcoma (KS)-associated herpesvirus, is a gamma human herpesvirus with a restricted geographic distribution (Sarid et al., 1999). Similar to the EBV, most infections with HHV8 remain asymptomatic. Nevertheless, the oncogenic potential for HHV8 infection is suggested by its association with all types of KS (Chang Y et al., 1994; Moore and Chang, 1995; Buonaguro et al., 1996), with primary effusion or body cavity-based lymphomas (PEL or BCBL) (Cesarman et al., 1995), and with multicentric Castleman disease (MCD) (Soulier et al., 1995). However, many aspects of the natural history, epidemiology, and pathogenesis of HHV8 remain uncharacterized.

Morphologically, HHV8 resembles other herpesviruses. A core of linear double-stranded DNA is surrounded by a capsid, a protein-rich tegument, and an outer lipid envelope (Renne et al., 1996a; Wu L et al., 2000). The HHV8 genome is approximately 165 kb in size and encodes more than 85 viral proteins (Renne et al., 1996a; Russo et al., 1996). Many HHV8-encoded genes are oncogenes that are either homologues of human oncogenes or unique to HHV8 or the rhadinovirus species (reviewed by Moore and Chang, 2003). Though the HHV8 genome is generally highly conserved, five major variants (genotypes A through E) have been characterized to date, which have restricted geographic distributions (Zong et al., 1999; Biggar et al., 2000). The five genotypes have no apparent specific disease associations (Hayward, 1999).

HHV8 was discovered by the creative application of representational difference analysis in 1994 in lesions from KS tumors (Chang Y et al., 1994) and is the first known human gamma-two herpesvirus, or rhadinovirus (Moore et al., 1996; Russo et al., 1996). HHV8 is closely related to several rhadinovirus species that infect nonhuman primates. The EBV, a gamma-one herpesvirus, is the closest relative to HHV8 among human herpesviruses (Moore et al., 1996; Russo et al., 1996).

Although HHV8 is detectable in the malignant spindle cells from KS lesions, cultured KS cells lose their HHV8 infection after only a few passages (Sarid et al., 1999). In addition, HHV8 is difficult to transmit to primary cells in culture (reviewed in Ablashi et al., 2002). Thus, little is known about the effect of HHV8 on untransformed cells. In one primary cell line, successful infection with HHV8 resulted in the development of a spindle-cell morphology, typical of the malignant cell type in KS lesions (Cannon et al., 2000; Wu et al., 2001). The cells also lost contact inhibition and expressed the virus-encoded latent nuclear antigen (LANA-1 and LNA-1). In addition, approximately 10% of the spindle-shaped cells were observed to spontaneously enter the lytic cycle and express viral structural proteins (Cannon et al., 2000; Wu et al., 2001). In contrast, in a previously transformed cell line, latent HHV8 infection was maintained indefinitely after transmission, although the cells could be induced to enter the lytic cycle (Moses et al., 1999). Transition to lytic replication resulted in the development of the spindle-cell morphology in these cells.

**Natural History of HHV8 Infection**

According to serologic studies, sexual intercourse and nonssexual intrafamilial transmission appear to predominate in the spread of HHV8. Sexual transmission is particularly efficient among male homosexuals, although no clear pattern of specific high-risk homosexual behavior has emerged to date (Schulz et al., 2002). HHV8 DNA has been detected in semen samples, but only rarely or in small quantities, and thus it is unclear whether semen is a relevant mode of exposure during sexual activity (Schulz et al., 2002). Close personal contact and saliva are likely routes of non-sexual intrafamilial transmission (Ablashi et al., 2002). Of interest, seropositivity for HHV8 was associated with HBV infection among Ugandan children and adolescents, suggesting that factors that favor HBV transmission may also increase exposure to HHV8 (Mayama et al., 1998). Perinatal transmission does not appear to occur frequently (Goedert et al., 1997; Mayama et al., 1998), but HHV8 appears to spread non-perinatally from mother to child and between siblings in populations of moderate to high endemicity (Gessain et al., 1999; Plancoulaine et al., 2004). In one endemic population, the risk of intrafamilial HHV8 transmission was related to the relatives' HHV8 serostatus, but not to anti-HHV8 titer (Plancoulaine et al., 2004). Parenteral exposure and solid organ transplantation confer a low risk of HHV8 transmission (Panavicini et al., 1997; Engels et al., 1999; Pellet et al., 2003; Renwick et al., 2002).

HHV8 appears to establish a lifelong persistent infection in the host, consisting of other herpesviruses (Ariyoshi et al., 1998; Biggar et al., 2003). In vitro studies suggest that heparan sulfate is the cellular receptor for HHV8 (Birkmann et al., 2001). Subsequent entry into the cell appears to occur by clathrin-mediated endocytosis (Akula et al., 2003). In vivo, the circulating cell types most frequently infected with HHV8 are B-lymphocytes and monocytes (Monini et al., 1999).
In latently infected cells, HHV8 DNA exists as a circular episome in the cell nucleus (Renne et al., 1996a). Only a small subpopulation of infected cells undergoes lytic replication at any given time; in latently replicating cells, the viral genome is linear (Renne et al., 1996a; Orentsen et al., 1997). Very few HHV8 genes are expressed in the latent phase (Renne et al., 1996b; Zhong et al., 1996). These include LANA-1 or LNA-1 and K12 or Kaposin. The proteins encoded by these and other constitutive latent cycle genes have cell-transforming capabilities and other complex functions (Ablashi et al., 2002). A second latent nuclear antigen, LANA-2, is expressed in PEL and MCD but not in KS lesions and is a potent inhibitor of programmed cell death (Rivas et al., 2001). A broader array of HHV8 genes is expressed in the lytic cycle, including capsid proteins and envelope glycoproteins involved in viral replication (Sarid et al., 1999; Schulz et al., 2002).

Host control of HHV8 replication is likely to depend heavily on cellular immunity. Consistent with this assumption, CTLs, specific for the latent K12 protein, as well as for several envelope glycoproteins and other structural antigens, have been documented in HHV8-seropositive subjects (Osman et al., 1999; Wang et al., 2001). Natural killer (NK) cells may also contribute significantly to the control of latent HHV8 (Sirianni et al., 2002). In addition, lytic cycle proteins and LANA-1 are specific targets of circulating antibodies, although it is unknown whether the humoral response further enhances overall control of HHV8 replication. The LANA-2 antigen does not appear to be immunogenic (Rivas et al., 2001).

Based on limited data from seroconverters to HHV8 antibody positivity, primary infection with HHV8 leads to the appearance of HHV8 DNA in PBMCs, and of extracellular serum viremia (Goudsmit et al., 2000; Wang et al., 2001). Ex vivo immunologic experiments suggested that this primary infection elicits a lytic antigen-specific CTL response and increased secretion of the cytokine IFN-γ, and that such responses may occur several months prior to the appearance of detectable circulating HHV8 antibodies (Wang et al., 2001). The observed lag between the cellular response and detection of HHV8 antibody may indicate that initial titers are very low, close to detectable limits of available serologic assays (Biggar et al., 2003). It is unknown whether this phenomenon, especially among HIV-infected men, for subjects to mount antibodies to only one (i.e., lytic or latent) antigen at seroconversion and to develop a broader humoral response over subsequent months to years (Goudsmit et al., 2000; Biggar et al., 2003).

Overall, antibody reactivity is more commonly observed to lytic than to latent HHV8 antigens. No severe clinical illness has been described in association with primary HHV8 infection, although limited data are available. Among five HIV-negative male seroconverters, four reported transient and nonspecific symptoms, such as localized rash, lymphadenopathy, and fatigue around the estimated time of seroconversion (Wang et al., 2001). Infection appeared to occur without clinical signs or symptoms in the fifth man. Similarly, low-grade fever was a common manifestation in immunocompetent infants and children with presumed primary HHV8 infection (Andreoni et al., 2002). A skin rash, respiratory tract infection, and/or cervical lymphadenopathy were also observed in some of the children. Though based on small numbers, these reports do not suggest a difference between children and adults in the experience of primary infection.

The nature of host–virus interactions after primary infection with HHV8 is poorly understood, and serologic profiles associated with viral reactivation are not characterized. A pattern of increasing HHV8 antibody titers precedes the diagnosis of KS in a subset of subjects (Renwick et al., 1998; Jacobson et al., 2000; Biggar et al., 2003). However, duration of HHV8 infection also predicts increasing titers of antibody seropositivity (Plancoulaine et al., 2000). It is not uncommon, especially in endemic areas (Wojcicki et al., 2004; Mbuliaye et al., 2005).

Regions of low endemicity include the United States, the United Kingdom, and much of Northern Europe and Asia (Schulz et al., 2002). Reported general population seroprevalences typically range from 2% to 5% in the United States and between 3% and 5% in Northern Europe and Southeast Asia, depending on the serologic assay used. In contrast, intermediate levels of endemicity characterize Mediterranean populations, such as Italy and Greece, where prevalences of 14% to 30% have been reported. In Italy, reported seroprevalences are usually high in southern areas but closer to those of nonendemic populations in the North, in parallel with the distribution of “classic” (HIV-negative) KS (Calabro et al., 1998; Whitby et al., 1998). HHV8 infection is considered highly endemic in parts of Africa, where the greatest population seroprevalences have been documented. For example, some countries in sub-Saharan Africa have reported seroprevalences of 50% to 60% (Schulz et al., 2002).

HHV8 Biomarkers

Molecular biomarkers for HHV8 infection include HHV8 DNA, which is consistently demonstrable in KS and PEL specimens by PCR, in situ hybridization, Southern blot hybridization, and immunohistochemistry (Ablashi et al., 2002). Two HHV8 RNA transcripts can also be detected using in situ hybridization: T0.7 which likely encodes a latent protein and is expressed in resting tumor cells; and T1.1, which appears to be an untranslated transcript expressed exclusively during lytic infection (Sarid et al., 1999). These molecular technologies have been invaluable to studies of the virology and biology of HHV8. For epidemiologic investigations, however, these techniques are not as informative, due in part to the low prevalence (often <10%) of HHV8 DNA detected in asymptomatic HHV8-seropositive subjects (Whitby et al., 1995; Engels et al., 2000).

Alternatively, a variety of serologic methods have been used in epidemiologic studies to detect antibody to HHV8. The most common approaches include IF-based assays, (Kedes et al., 1996; Lennette et al., 1995; Engels et al., 1996), and ELISA (Chatlynne et al., 1996), and EIA (Chatlynne et al., 1996). The IFAs typically test serum reactivity to PEL cell lines that harbor HHV8 but not EBV or HIV (Ablashi et al., 2002). ELISA and EIA methods incorporate single or multiple recombinant
HHV8-encoded antigens or whole virus lysate (Chandran et al., 1998; Chatlynne et al., 1998; Simpson et al., 1996).

The earliest assays demonstrated poor reproducibility, especially among healthy blood donors, in an interassay comparison analysis (Rakbin et al., 1998). More recently, developed tests have shown improved interassay reproducibility (Engels et al., 2000). In the absence of a gold standard of true HHV8 status, these serologic assays cannot be directly evaluated for accuracy. Furthermore, the methods are not standardized, which can complicate the comparison of results across different study populations. Of interest, a study comparing six laboratories that used different multi-assay algorithms to determine HHV8 serostatus reported improved but nonetheless variable inter-laboratory agreement (Pellett et al., 2003). The use of assays to detect both antilytic and antilatent antibodies is generally recommended for improved sensitivity and specificity of HHV8 antibody detection. Clearly, further development of serologic assays is necessary to facilitate the epidemiologic study of HHV8.

The Major HHV8-Associated Malignancies

Kaposi Sarcoma

Four variants of KS are currently recognized, which are histologically identical but differ somewhat in their typical epidemiologic and clinical characteristics (Tapper et al., 1993). HHV8 is now considered to be a necessary cause of all four forms of KS (Moore and Chang, 1998). However, KS is a rare outcome of HHV8 infection, and thus co-factors must cooperate with HHV8 in KS pathogenesis.

“Classic” or “sporadic” KS is the condition first characterized by Moritz Kaposi in 1872. Patients present with a pigmented skin lesion of multiple nodules and plaques that appear first on the lower limbs and may spread to arms, face, and torso over a period of years to decades. In a minority of cases, the lesions progress to involve visceral mucosal surfaces (Antman and Chang, 2000). Classic KS is rare and is diagnosed primarily in elderly men of Mediterranean or Eastern European origin. Its occurrence has been attributed in part to immune suppression related possibly to age, host genetics, a history of other cancer, or to regional factors such as malaria (Kaloterasakis et al., 1995; Calabro et al., 1998; Iscovich et al., 1999). Recent evidence from both epidemiologic (Touloumi et al., 1999; Goedert et al., 2002) and laboratory in vitro studies (Fiorelli et al., 1998; Monini et al., 1999) appears to contradict this hypothesis by suggesting that immune activation and inflammation predict classic KS, although these immunologic states may not be mutually exclusive. Of interest, cigarette smoking, which has immune-suppressive effects, has been found to be inversely associated with risk of classic KS (Goedert et al., 2002; Hatzakis A, personal communication). Of note, chronic inflammation was also implicated as a determinant of HHV8 viral load among HHV8-seropositive (Goedert et al., 2002).

Of note, HHV8-seropositive subjects who use topical steroids may also have an elevated risk, although it could not be determined whether the association reflected an effect of the underlying dermatitis or of the steroid use itself. Regardless, this association suggests that localized immunologic events in the skin also contribute to KS pathogenesis (Goedert et al., 2002). In contrast to EBV models, age and route of exposure to HHV8 does not appear to modulate risk of progression to classic KS (Goedert et al., 2002). These findings warrant confirmation in other HHV8-seropositive populations.

A second KS variant, called “endemic” or “African” KS, is so named because of its high prevalence in sub-Saharan Africa. For decades, endemic KS has comprised a substantial proportion of all tumors diagnosed in some Central African countries, such as Uganda and Zambie (de-Thé et al., 1999; Antman and Chang, 2000). Endemic KS affects HIV-negative adults and children, in contrast to other variants. In adults, the condition is more common in men than in women and follows a relatively indolent clinical course, similar to classic KS. In children, however, the disease often involves lymph nodes in addition to the typical cutaneous sarcoma lesions and is more aggressive (Ziegler and Katongole-Mbidde, 1996). Predictors of endemic KS risk among HHV8-seropositive persons are not well characterized. Factors indicative of relative affluence were associated with an elevated risk of endemic KS in Ugandan cancer patients, suggesting a role for delayed age at primary infection with HHV8 (Ziegler et al., 2003). It is noteworthy that rarely ever having worked in the field was one of the lifestyle factors associated with endemic KS in the latter population; the practice of going barefoot likely reflects active farming and is therefore not inconsistent with an affluence-related effect on KS risk (Ziegler, 2003). This effect may reflect localized immune suppression of the skin due to blockage of the lower lymphatic system by fine soil particles, which can pass through the barefoot skin. If confirmed, this finding would appear consistent with the reported association of topical steroid use with classic KS (Goedert et al., 2002) and would thus expand the evidence that localized immune dysregulation of the skin is important in KS pathogenesis.

A third variant, “iatrogenic” KS, occurs in patients undergoing immunosuppressive therapy after organ transplantation or for a variety of other conditions. KS is a rare outcome of iatrogenic immune suppression (Antman and Chang, 2000) but is diagnosed more frequently among HIV-I-seropositive men (Nawar et al., 2005). Table 26-4 summarizes the major risk factors that have been reported for KS. However, some patients have been reported to regress upon withdrawal of immunosuppressive therapy (Sarid et al., 1999). The latter observation is compelling evidence that immune suppression is a significant risk factor for KS development. Thus, immune activation and immune suppression may each influence the natural history of HHV8 infection in some undescribed manner (Touloumi et al., 1999).

The fourth KS variant, known as “epidemic” or AIDS-associated KS, occurs in HIV-positive persons, primarily in homosexual men, and is the most prevalent type of KS in many countries. Epidemic KS can also be the most clinically aggressive and disseminated type, affecting lymph nodes and visceral mucosal tissues as well as the skin (Sarid et al., 1999). There is strong epidemiologic evidence for an association of HHV8 with AIDS KS. Serocversion to HHV8 antibody positivity both precedes and predicts development of AIDS-KS, which often occurs within 5 to 10 years in HIV-positive homosexual men (Renwick et al., 1998; Jacobson et al., 2000). The rate of progression to KS was substantially shorter when HIV-1 infection preceded seroconversion to HHV8 (Renwick et al., 1998; Jacobson et al., 2000), which may reflect promotion of KS development by HIV-related immune suppression. The latter interpretation is consistent with the findings that markers of more advanced immune suppression, including decreased CD4+ cell count (Renwick et al., 1998; Jacobson et al., 2000) and increased HIV-1 viral load (Jacobson et al., 2000), were independent predictors of KS in these subjects. However, Nawar and colleagues (2005) recently reported an apparent protective effect of cigarette smoking on KS risk among HHV8-seropositive homosexual men with AIDS, consistent with prior observations in classic KS and with a role for immune activation in epidemic KS etiology. The activation of HHV8 by the HIV-1 Tat protein is also believed to directly affect the proliferation of HHV8-infected cells, further enhancing HIV-1-associated risk of KS (Ariyoshi et al., 1998; Fiorelli et al., 1998). In addition, as suggested for endemic KS, relative affluence may increase the risk of AIDS-KS in HHV8- and HIV-1-seropositive men (Nawar et al., 2005). Table 26-4 summarizes the major risk factors that have been reported for KS. However, some of these associations are based on sparse data and require confirmation.

Collectively, the observed predictors of the four types of KS suggest that immune dysfunction is a central factor in the pathogenesis of KS. Consistent with this view, diminished CTL responsiveness to HHV8 antigens is associated with KS pathogenesis (Osman et al., 1999), whereas restored NK cell cytotoxicity appeared to explain KS regression in AIDS patients successfully treated with highly active antiretroviral therapy (Sirianni et al., 2002). However, some of the
immunity-related evidence appears contradictory, implicating immune activation in KS pathogenesis. Emerging evidence from in vitro studies suggests an important role for inflammatory cytokines and in particular for IFN-γ in the early development of KS (Fiorelli et al., 1998; Monini et al., 1999). It is not clear how inflammatory and immune suppressive forces interact to result in KS nor is it known how HHV8 gene expression contributes to pathogenesis. To date, the characterization of HHV8-encoded genes suggests a partial overlap in the viral strategies for immune escape and induction of cell proliferation and thus offers unique insights into the interrelationship between viral immune evasion and tumorigenesis (Moore and Chang, 2003). These issues warrant further investigation.

Primary Effusion Lymphoma

Primary effusion lymphoma (PEL) is a rare subtype of NHL that is characterized by detection of HHV8 DNA as well as other highly stringent diagnostic criteria (Cesarman et al., 1995, 1996). In brief, the malignancy is a lymphomatous effusion of apparent B-cell origin occurring in the pleural cavity and pericardium, with no discernable tumor mass. The vast majority of cases occur in AIDS patients, some-times as a second malignancy after KS. The presence of HHV8 distinguishes PELs from other body-cavity-based lymphoma types (Ablashi et al., 2002). Most PEL lesions also harbor EBV. The pathogenic role of HHV8 and EBV in PEL is not known, although expression of viral oncogenes is likely involved (Ablashi et al., 2002). The rarity of PEL even in HHV8-seropositive persons, however, suggests that strong cofactors affect the risk of this malignancy.

Multicentric Castleman Disease

Multicentric Castleman disease (MCD) is the plasma cell subtype of Castleman disease, an atypical lymphoproliferative disorder that is poorly understood (Ablashi et al., 2002). MCD is considered a polyclonal, non-neoplastic disorder but is associated with immune dys-regulation and with a high risk of malignancy, and in particular of KS and NHL. MCD occurs more commonly in older persons and in men. In AIDS-associated MCD, nearly all cases harbor HHV8, as do approximately half of the HIV-negative cases of MCD (Soulier et al., 1995; Ablashi et al., 2002). The role of HHV8 in MCD pathogenesis is not understood but may include an effect of the HHV8-encoded functional homologue to the cytokine IL-6, a potent B-cell growth factor. Both human and viral IL-6 (vIL-6) are expressed in the lymphoid tissues of MCD patients.

Other Associations

Rettig and colleagues (1997) created a stir when they reported finding HHV8 DNA by PCR in bone marrow stromal cells from 15 of 15 patients with multiple myeloma (MM). Specimens from two of eight patients with monoclonal gammapathy of uncertain significance (MGUS), a nonmalignant condition with a high risk of progression to MM, were also PCR-positive for HHV8 genome (Rettig et al., 1997). Because the cytokine IL-6 is an important growth factor for MM, and HHV8 encodes vIL-6, Rettig et al. (1997) proposed that HHV8 in the bone marrow microenvironment contributes to plasma cell transformation by paracrine secretion of vIL-6. Despite the biological plausibility of an association of HHV8 with MM, however, the majority of epidemiologic evidence does not support a link between HHV8 and MM (Ablashi et al., 2002).

In addition to MM, HHV8 DNA was reportedly detected in diagnostic tissue specimens from a variety of diseases, including pemphigus, bullous pemphigoid, a variety of skin tumors, sarcoidosis, and Kikuchi disease. However, these reports have been disputed in the literature, and the collective evidence remains either inconclusive or not supportive of a causal role for HHV8 in the pathogenesis of these diseases (Ablashi et al., 2002).

Prevention and Future Research

With inadequate knowledge of the routes of transmission of infectious HHV8, it is difficult to propose a comprehensive strategy to prevent infection. Nevertheless, an important component of such a strategy is clearly the avoidance of behaviors that increase the risk of HIV-1 infection. The possibility of interventions to prevent nonsexual modes of transmission of HHV8 are less certain, although it has been suggested, based on what is understood of the natural history of KS, that a vaccine and immunotherapies are worthy of exploration (Sarid et al., 1999).

Therefore, a more precise understanding of routes of sexual and nonsexual horizontal transmission of infectious HHV8 virions remains an important focus for future research. The related question of host factors that confer susceptibility to infection, once exposed, also warrants study.
In particular, studies of the host response to primary infection, including the impact of age and route of initial infection, would contribute valuable information. The serologic profiles pertaining to HHV8 reactivation during latent infection also warrant characterization, as do temporal patterns and determinants of viral load. Given the implied roles for both immune suppression and immune activation in KS pathogenesis, prospective epidemiologic studies that incorporate biomarkers of each of these processes will be valuable for clarifying the immunologic parameters that predict risk of KS. The opportunity to conduct these proposed studies will be heavily dependent on applications of biotechnology that permit more effective quantification of serum HHV8 antibodies and viral load. Perhaps in part because the clonality of KS lesions has been long disputed (Ablashi et al., 2002), few studies have addressed whether HHV8 itself is clonal within a KS lesion as well as among KS lesions in a given patient. The limited data available suggest that many KS lesions contain either oligo- or monoclonal HHV8 (Judd et al., 2000; Gessain and Duprez, 2005). These data are consistent with an early etiologic role for HHV8 in KS pathogenesis and with the view that KS progresses from a polyclonal disease to a monoclonal malignancy (Gessain and Duprez, 2005). Further evaluation of HHV8 clonality may thus help to resolve the debate over the clonality of KS lesions and contribute important insights into the pathogenesis of HHV8-related malignancies.

The informativeness of the future epidemiologic studies of HHV8 will likely be amplified by continuing advances in the areas of molecular biology, virology, and immunology. Indeed, multidisciplinary approaches to resolving the mysterious aspects of HHV8 infection are especially warranted. As a more comprehensive understanding of HHV8 natural history and pathogenesis emerges, the opportunity to develop effective preventive measures, including appropriate public health messages and immune-based preventive therapies, to reduce the global burden of KS and associated diseases may be substantially enhanced.

HUMAN PAPILLOMAVIRUSES

Papillomaviruses cause warts (papillomas) that are either flat or raised. These ubiquitous viruses have evolved slowly with their animal hosts, and the infections are species-specific. Papillomavirus infections are virtually always benign. However, persistent infections with oncogenic genotypes of human papillomaviruses (HPVs) cause almost all cases of cervical cancer and a smaller fraction of other cancers, totaling a half million cases per year worldwide (Pisani et al., 2002; Bosch and de Sanjose, 2003). This brief review will describe the epidemiologic and natural history of HPV infection and invasive cervical carcinoma was first demonstrated in 1907. The person-to-person infectivity and latency of genital HPV infections was established by the incidence of genital warts in women, 4 to 6 weeks after the return of their husbands (who had new penile warts) from military service (Barrett et al., 2003). Studies of HPV and cancer have predominantly addressed cervical neoplasia because of its high prevalence. The relationship of cervical carcinoma to sexual behavior, particularly lifetime number of sexual partners, has been suspected for over a century and well-established by epidemiologic studies since the 1960s (Brinton and Fraumeni, 1986). Advances in HPV-cancer cytopathology occurred over the last half of the 20th century as various groups described the preinvasive abnormalities related, on one hand, to warts and HPV, and on the other hand, to risk of subsequent invasive cervical cancer (Meisels, 1969; Richart and Barron, 1969; Koss, 1989). The molecular biological association of HPV infection and invasive cervical carcinoma was first demonstrated by zur Hausen and colleagues (2002) who found HPV DNA of previously uncharacterized types (now called HPV 16 and HPV 18) in cervical carcinomas. The early molecular epidemiologic studies of HPV in the 1980s established the plurality of HPV types and the association of specific types with different tissues and degrees of neoplasia (Fuchs et al., 1988; Lorincz et al., 1992).

Natural History of HPV Infection

All HPV infections are usually transmitted by person-to-person, skin-to-skin contact, as supported by the characteristic age-specific prevalences of outcomes of different HPV infections; for example, common cutaneous warts in young children (Vittorio et al., 1995). Male and female genital HPV infections (as measured by DNA, cytologic diagnosis, or presentation of overt genital warts) peak in early adulthood concurrent with the age of usual onset of sexual intercourse (Young et al., 1992a; Tjan et al., 2002). The appreciable prevalence of another genital HPV infection, as measured by DNA, in women reporting only one lifetime sexual partner, mainly reflects their partners' sexual experiences with other partners (Castellsague et al., 2002). As a rule, and clinical manifestations. Each of these groups is composed of several species (α, β, ...). Within species different types (not "strains") are defined based on proportion of DNA sequence homology (de Villiers, 2001; Schiffman et al., 2005). Different types share less than 90% homology in the L1 region, which tends to be genetically conserved. HPV types are numbered chronologically in order of characterization, not relatedness. There are considerably more than 100 HPV types, of which approximately 90 have already been characterized and assigned numbers (de Villiers, 2001). The process of finding and characterizing novel HPV types continues; however, the types now being identified are mainly commensal and do not contribute meaningfully to cancer burden, with the possible exception of skin cancer. Within HPV types, even finer evolutionary branchings, called variants, have been defined and associated with oncogenicity (Yamada et al., 1997; Hildesheim et al., 2001; Xi et al., 2002).

Epidemiologists make use of the genetic species of HPV to permit analyses that lack statistical power if each type were considered separately. Alternatively, the associations are presented for groupings of HPV types and disease. Most mucosal types cause benign flat lesions or no discernible pathology and are termed "low risk." A few mucosal types (HPV 6 and 11 primarily) cause benign, exophytic, anogenital warts called condyloma acuminatum (Wiley et al., 2002). The proven oncogenic HPV types are in the mucosal group but not all in one clade. The Φ9 and Φ7 species, containing HPV 16 and HPV 18, respectively, are the most important. The oncogenic types include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and possibly types 26, 73, and 82 (Bosch et al., 1995; Munoz et al., 2003; Cogliano et al., 2005). HPV 16 is uniquely prevalent and carcinogenic and causes half of the cancer burden (Bosch and de Sanjose, 2003).

Animal and human papillomavirus-related lesions have been recognized for more than a millennium (Bruns, 1992). In the twentieth century, cottontail rabbit and bovine papillomaviruses provided the most important animal models of papillomavirus infections and viral carcinogenesis (Lancaster and Olson, 1982). Transmission of human warts by cell-free filtrate was first demonstrated in 1907. The person-to-person infectivity and latency of genital HPV infections was established by the incidence of genital warts in women, 4 to 6 weeks after the return of their husbands (who had new penile warts) from military service (Barrett et al., 2003). Studies of HPV and cancer have predominantly addressed cervical neoplasia because of its high prevalence. The relationship of cervical carcinoma to sexual behavior, particularly lifetime number of sexual partners, has been suspected for over a century and well-established by epidemiologic studies since the 1960s (Brinton and Fraumeni, 1986).

Advances in HPV-cancer cytopathology occurred over the last half of the 20th century as various groups described the preinvasive abnormalities related, on one hand, to warts and HPV, and on the other hand, to risk of subsequent invasive cervical cancer (Meisels, 1969; Richart and Barron, 1969; Koss, 1989). The molecular biological association of HPV infection and invasive cervical carcinoma was first demonstrated by zur Hausen and colleagues (2002) who found HPV DNA of previously uncharacterized types (now called HPV 16 and HPV 18) in cervical carcinomas. The early molecular epidemiologic studies of HPV in the 1980s established the plurality of HPV types and the association of specific types with different tissues and degrees of neoplasia (Fuchs et al., 1988; Lorincz et al., 1992).

Natural History of HPV Infection

All HPV infections are usually transmitted by person-to-person, skin-to-skin contact, as supported by the characteristic age-specific prevalences of outcomes of different HPV infections; for example, common cutaneous warts in young children (Vittorio et al., 1995). Male and female genital HPV infections (as measured by DNA, cytologic diagnosis, or presentation of overt genital warts) peak in early adulthood concurrent with the age of usual onset of sexual intercourse (Young et al., 1992a; Tjan et al., 2002). The appreciable prevalence of another genital HPV infection, as measured by DNA, in women reporting only one lifetime sexual partner, mainly reflects their partners' sexual experiences with other partners (Castellsague et al., 2002). As a rule,
Epidemiology of Infection

Accurate estimates of the prevalence and incidence of HPV infection must be referred to a particular epithelial tissue, a particular HPV type or group of types, a specific specimen collection and testing protocol, and a defined population. Nonetheless, HPV is the most common sexually transmitted infection, and tens of millions of sexually active individuals have been infected at some time with at least one type of oncogenic HPV (Franceschi et al., 2002). Although infection is relatively benign it is a persistent infection that raises concern.

However defined, the prevalence of oncogenic cervical types in a population depends on the age and sexual practices of the population (Bauer et al., 1993). In general, young sexually active individuals appear to experience the highest prevalence of oncogenic anogenital HPV infections, consistent with an "epidemic curve" after first sexual exposure. Again, HPV infections of the cervix have been best studied (Fig. 26-6). The drop in cervical HPV prevalence in women past their 20s is likely due to clearance or suppression of existing infections, combined with less exposure to new HPV types because of fewer new sexual partners. However, persistent infections increase with age (Castle et al., 2005). Of note, the point prevalence of HPV DNA in surveys of immunocompetent (HIV-uninfected) prostitutes is not always elevated, suggesting immunity in some women following intense exposure (Kjaer et al., 2000).

Although it is unwise to think in terms of a simple summary estimate of anogenital HPV prevalence without reference to age and sexual behavior, oncogenic HPV DNA prevalences of 15–30% are typical, using sensitive detection methods, with an annual incidence of 10% or more among sexually active young women (Franco et al., 1999; Sellors et al., 2003). HPV 16 is the most common type in health and disease, perhaps because it persists effectively (Herrero et al., 2000; Munoz et al., 2003). The prevalence of concurrent infections with multiple types of HPV typically approaches 20–30% of infected women, using the best PCR methods (Herrero et al., 2000). It is not clear whether HPV types influence each other's presence either directly or via the host immune system, but the available evidence suggests that each HPV type can be viewed as a separate infection with its own natural history (Thomas KK et al., 2000; Liaw et al., 2001).

HPV infects the anogenital epithelium broadly. Vaginal and vulvar (introital) HPV infections are just as common and varied in type completely virginal women are not infected at the cervix, although nonintromissive sexual behaviors can lead to anogenital transmission (Winer et al., 2003). Autoinoculation and spread appears to be possible. Nonsexual transmission of anogenital infection is probably uncommon, although the point remains controversial (Dillner et al., 1999; Rice et al., 1999). Fomite transmission is possible but probably uncommon (Strauss et al., 2002). Vertical transmission of HPV types 6 or 11 during vaginal birth rarely cause laryngeal papillomas, which can be serious if they threaten airway obstruction (Silverberg et al., 2003).

HPV infection can lead to a wide range of epithelial response, from no apparent lesion to flat or raised warts to invasive carcinoma. There is no known viremia or infection of nonepithelial tissues. Tissue effects are discussed more fully in other chapters as they relate to particular malignancies. The spectrum of HPV-related epithelial abnormalities leading to cancer was formerly considered a stepwise progression of increasingly severe intraepithelial neoplasia: grade 1 to grade 2 to grade 3 (including carcinoma in situ) to cancer (Richart and Barron, 1969). More recently, the biological meaning of this microscopically evident pathologic continuum has been questioned (Schiffman and Kjaer, 2003) and, for epidemiologic studies, it is more reliable and analytically useful to broadly classify tissue effects as acute HPV infection, with or without, associated lesions (which can be pathognomonic but are more often variable), precancer, or cancer.

Acute HPV infections, even with potentially oncogenic, mucosal types, are usually self-limited. Cervical HPV infections, as defined by DNA detection, usually disappear within 2 years of first incident appearance (Richardson et al., 2003). However, for already prevalent infections, defined at cross-sectional screening, the median time to disappearance is approximately 1 year (Ho et al., 1998).

The key immunity involved in the clearance of HPV infections is cell-mediated (type 1) response (Kadish, 2001; Berry and Palefsky, 2003; Pinto et al., 2003). Multiple warts of a single kind tend to regress concurrently, associated with an infiltration by antigen-presenting cells and lymphocytes. HIV/AIDS and iatrogenic immunosuppression, as part of organ transplantation, provide important insights into the effects of immunosuppression on HPV infection (Palefsky and Holly, 2003). These examples have established an association between immunosuppression, HPV infection, and anogenital precancerous lesions. An effect of immunosuppression on the rate of progression to invasive carcinoma is less clear. Recent data from two HIV cohorts of women indicate that immune status, as measured by CD4 counts, is more weakly linked to the detection of prevalent and incident HPV 16 cervical infection than other HPV type cervical infections (Strickler et al., 2003). These data suggest that HPV 16 is unique in avoiding immune surveillance and may help explain why HPV 16 is the most persistent HPV type, and the one linked to about half of cervical cancers worldwide. Antibodies against the HPV are detected in some, but not all, infected women (Wideroff et al., 1999; Carter et al., 2000).

HPV persistence, lasting for years, has been directly linked in cohort studies to the development of precancer (Ho et al., 1995; Nobbenhuis et al., 1999). Precancer develops in <10% of women with point prevalent oncogenic HPV infection; in comparison, the risk of development of precancer among women with oncogenic HPV infections that persist for 5 years or more is much greater (Schiffman et al., 2005). The sine qua non of oncogenic types is that they produce precancer when they persist, whereas low-risk types rarely do. As an important etiologic clue, HPV 16 persists longer than other HPV types and also is most likely to cause precancer when it does persist; the absolute risk at 5 years approaches 40%.

Cancer can be considered the most serious known outcome of HPV infection. Cervical cancer is by far the most common HPV-related cancer but still is rare compared to infection. The proportion of other anogenital cancers and oropharyngeal cancer attributable to HPV infection is lower than for cervical cancer, that is, they may be more multifactorial than cervical cancer (Herrero, 2003; Schiffman and Kjaer, 2003). The possible role of poorly defined cutaneous types of HPV in skin cancer etiology (Pfister, 2003) is discussed in the chapter on skin cancer.
as cervical HPV (Winer et al., 2003). Common HPV-induced penile lesions can be found in the urethral meatus, on the glans and shaft of the penis, and on the scrotum (Barraso, 1992; Bleeker et al., 2002; Franceschi et al., 2002; Baldwin et al., 2003). The available data suggest that penile HPV prevalence is comparable to cervical prevalence. Anal HPV infection, linked to an increased risk of anal neoplasia, is also extremely common in both sexes, particularly, but not at all exclusively, among individuals who practice intimate anal intercourse (Frisch et al., 1997; Palefsky et al., 1998).

Large-scale HPV DNA studies began in the 1980s and, thus, there are no long-term data addressing historical trends. Epidemiologists would like to relate the predictors of HPV prevalence to the known demographic risk factors for cervical cancer. Long-noted correlations within the United States of cervical cancer with religion (e.g., decreased rates in Jewish women), race (increased rates in African Americans), SES (increased rates in poorer women), and occupation (husbands away from home) are concordant with the known predictors of HPV as a sexually transmitted infection (Bauer et al., 1993). However, decades after the "sexual revolution," HPV is now so prevalent that all women with even a few sexual partners have a substantial risk of exposure. The historical epidemiologic associations are blurring.

HPV infection is common globally, and its role in anogenital carcinogenesis does not vary by region (Bosch and de Sanjose, 2003; Munoz et al., 2003). However, interesting subtle variations occur. The geographic distribution of HPV DNA detection has been studied mainly in correlation with cervical cancer incidence rates, and some expected ecologic associations have been seen (Bosch and de Sanjose, 2003). In many study populations, the prevalence of current HPV infection, as measured by DNA or cytology, decreases strongly with age as it does in the United States, from a peak at 15–25 years. But in other regions, there is no decrease or a secondary upturn at older ages is seen (Schiffman and Kjaer, 2003), a pattern that presents one of the remaining challenges to HPV epidemiologists (Fig. 26–6). Varying age trends in HPV prevalence might be rooted in differing societal sexual practices, immunologic senescence, and/or cohort effects. There is emerging epidemiologic evidence for each. (Castle et al., 2005).

HPV Biomarkers

After molecular biologists demonstrated in small studies that HPV DNA was present in cervical cancer tissue, it took several years to adapt HPV DNA measurement techniques to permit testing of noninvasively obtained cervical specimens collected at the time of pelvic examination by swab, scrape, brush, or lavage (Schiffman, 1992b). During the development phase, the adverse impact of HPV exposure misclassification on early studies of HPV and cervical neoplasia was so profound that it merits consideration by all epidemiologists using newly developed assays that are incompletely validated (Franco, 1992).

HPV infection is most often measured by HPV DNA detection, although the detectability of HPV DNA in a single specimen (point prevalence) clearly differs from lifetime exposure to HPV (cumulative incidence). The improved DNA measurement techniques now being used by most epidemiologists (Iflner and Villa, 2003) to assay all oncogenic types at once tend to yield roughly comparable results when performed expertly on adequate cervical cytologic specimens. Keratinized epithelial surfaces, such as the vulva, penis, or nongenital skin, have proven more difficult to test reliably.

Excluding in situ methods, which have rarely been used by epidemiologists, there are two categories of HPV DNA tests: those that identify nucleic acids directly, and those that amplify nucleic acids first and then detect the amplified product. In the first category currently is Hybrid Capture 2, or HC2 (Schiffman et al., 2000; Terry et al., 2001). The amplification methods currently used for HPV epidemiology are PCR-based (Castle et al., 2002a; van Dorn et al., 2002; Gravitt et al., 2003; Ifnner and Villa, 2003; Schiffman et al., 2005). Any data based on older techniques should be interpreted with caution. Very sensitive PCR techniques are available for research on extremely low levels of particular HPV types but have not been validated to test for all oncogenic types concurrently at a clinically relevant level.

HPV serologic assay development and validation have lagged behind advances in DNA testing (Wang and Hildesheim, 2003).

Lacking a source of abundant, native viral antigen, virus-like particles (VLPs) have been synthesized for many types and used particularly to test case-case series and archived, premorbid sera in case-control studies of invasive cancers, especially from the huge Scandinavian serology banks (Bjorge et al., 1997, 2002; Strickler et al., 1998; Mork et al., 2001; van Doornum et al., 2003). Seropositivity is a useful type-specific measurement of exposure, although 25–55% of infected women do not become persistently seroreactive (Wideroff et al., 1999; Carter et al., 2000). Newer, apparently more sensitive assays might improve this situation (Pastrana et al., 2004). HPV infections are limited to the epithelia and apparently induce weak immune responses. Though likely to be important, the mucosal antibody response to epithelial HPV infection is poorly understood.

Cervical Cancer

Case-control studies in dozens of countries have confirmed, with virtually no exceptions, the extremely strong association of oncogenic HPV DNA detection with risk of cervical cancer and intraepithelial precursor lesions (Bosch et al., 2002). More than 90% of cervical cancers and precancers contain HPV DNA, with the inclusion of "possible" infections raising the proportions even higher (Bohmer et al., 2003; Munoz et al., 2003). The cancer-associated types agree well with the relative transforming properties of the viral types as defined in vitro and with "phylogenetic" studies grouping HPV types by genetic relatedness. Case-control studies of HPV variants show, remarkably, that risk of precursor and cancer is associated with specific variants, reaching prevalence ORs in the thousands (Wang and Hildesheim, 2003).

The prospective evidence that oncogenic HPV infection precedes and predicts risk of cervical precursor and cancer is also strong and consistent (Wallin et al., 1999; Sherman et al., 2003). A single HPV measurement confers an increase in risk of approximately 10- to 30-fold over the following decade, whereas persistent oncogenic HPV infection is even more tightly linked to risk of precursor (Kjaer et al., 2002). HPV16 and HPV18 are particularly important carcinogens (Khan et al., 2005). In summary, persistent oncogenic HPV infection is virtually necessary for the development of precursor and cancer of the cervix (Bosch et al., 2002).

As discussed in the relevant chapters, the etiologic fractions of other anogenital cancers and oropharyngeal cancer due to HPV infection are lower but still substantial, although the available epidemiologic data for causality are weaker, partly because these cancers tend to be rare.

Biological Causal Mechanisms

The molecular biology of HPV and other papillomaviruses, as model DNA tumor viruses, is under intensive study. The reader is referred elsewhere for recent reviews, as only a few critical points can be mentioned here (Kadish, 2001).

It is assumed that the initial site of HPV infection is the germinal cells in the basal layer of the epithelium, secondary to minor epithelial injuries during sexual contact. HPV-induced lesions appear to be monoclonal, suggesting that each lesion derives from a single infected germinal cell (Park et al., 2003). Early viral transcripts are detectable in the basal and parabasal layers of the epithelium, whereas capsid production and virion assembly occur in the upper superficial layers of the differentiated epithelium (Stoler et al., 1994). In early HPV-induced lesions (typically destined to regress), squamous differentiation in the more superficial layers of the epithelium becomes abnormal, but the cells continue to differentiate, such that there is only a minimal effect on the expansion of the proliferative (nondifferentiated or "immortalized") compartment to approximately one-third or less of the full thickness of the epithelium. The accompanying expansion of the middle portion (spinous layer) of the epithelium characterizing HPV lesions, particularly condylomata, results from a reduced rate of squamous cell sloughing, rather than increased rate of cell turnover.

The E6 and E7 proteins are principally responsible for HPV neoplastic effects, via interactions with pRb and p53-related cell-cycle pathways, respectively (Munger and Howley, 2002; Fehrmann and Laimins, 2003). The specific details of these molecular interactions are
increasingly understood, making HPV an important model of viral carcinogenesis (Broker and Chow, 2001).

In most infections, the HPV genome is maintained in the cell nucleus in an episomal state. In the majority of invasive cervical carcinomas, however, integration of HPV DNA into the host genome is found (Ziegert et al., 2003). Integration tends to occur throughout the cell genome. However, with reference to the viral genome, integration is not random. In cancers, the E6 and E7 open reading frames are preserved, with frequent disruption during integration of the E1 and E2 genes that normally inhibit E6 and E7. Thus, continuous production of E6 and E7 proteins appears to have a role in HPV carcinogenicity and, in fact, the E6 and E7 regions of the HPV genome are transcriptionally active in HPV-associated cervical carcinomas and derived cell lines. RNA-based assays for possible clinical use are currently being validated.

Cofactors

For unknown reasons, HPV infection tends to cause cancer at boundary areas ("transformation zones") where one kind of epithelium contacts and sometimes gradually replaces another. The cervix, tonsils, and anus are examples of tissues with transformation zones prone to HPV carcinogenesis.

Apart from target tissue, a crucial current question is "What else besides HPV type predicts the risk of viral persistence and progression to precancer?" We hope eventually to study viral persistence and progression to precancer separately and prospectively, but that level of distinction has not yet been achieved. The three categories of factors that seem most likely to be responsible for persistence and progression include (1) viral factors, (2) environmental cofactors, and (3) host factors (Castellsague and Munoz, 2003).

Apart from the powerful importance of HPV type and variant, no other critical viral factors have been identified. It appears that very high levels of HPV DNA (often seen in acute benign warts) are not (except for HPV16) more closely linked to persistence and progression than are low levels, although extremely low levels probably reflect an increased tendency toward clearance (Lorincz et al., 2002).

Smoking is the environmental cofactor most consistently linked with persistence and progression of HPV infection (Castle et al., 2003b; Castellsague and Munoz, 2003). Smoking has both mutagenic and immunologic effects, and it is unclear which is primarily involved. Other possible environmental cofactors that interact with HPV include chronic inflammation, other sexually transmitted infections like Chlamydia trachomatis, and levels of antioxidant nutrients or folate (Castle and Giuliano, 2003). It is possible that sunlight and HPV interact in the etiology of skin cancer (Pfister, 2003).

With regard to host factors that interact with HPV, multiplicity increases the risk of progression to cervical precancer and cancer although the mechanism is unclear (Munoz et al., 2002, 2003). Parity might act by influencing immunity or by nutritional, traumatic, or even biochemical mechanisms (Gravitt and Castle, 2001). Long-duration hormonal contraceptive use may increase the risk of precancer and cancer (Moreno et al., 2002; Green et al., 2003; Smith et al., 2003) among HPV infected women if screening and treatment of intraepithelial precursor lesions does not intervene. There is some laboratory support for hormonal influences on HPV (de Villiers, 2003).

Prevention

Oncogenic, genital HPV transmission would be prevented by abstinence. However, even conscientious condom use would not entirely prevent the spread of genital HPV infections because genital HPV infections can involve the scrotum and perineum (Manhart and Koutsky, 2002). Ultimately, prophylactic HPV vaccines that induce neutralizing antibodies might permit primary prevention of HPV and its clinical sequelae, including cancers (Lowy and Frazer, 2003). At least two very large and highly promising Phase III efficacy trials of HPV 16 and 18 prophylactic vaccines are now underway, based on virus-like particle antigens produced in insect cells or yeast (Koutsky et al., 2002; Lowy and Frazer, 2003; Harper et al., 2004; Villa et al., 2005). Early therapeutic trials of HPV infection per se, to permit treatment of HPV-induced intraepithelial lesions and secondary prevention or even treatment of cervical cancer, are also underway at earlier phases (Eiblen et al., 2003).

Until polyvalent vaccines are available and widely used for multiple cohorts of women, HPV infections and HPV-induced lesions will still be highly prevalent (Sherman et al., 1998; Hughes et al., 2002). HPV control efforts will be aimed at preventing carcinoma, particularly cervical carcinoma. In the United States, the standard response to the detection of HPV-associated mild lesions is tending toward "watchful waiting" to permit clearance, whereas the treatment of precancerous cervical lesions is destruction of the lesion and the remaining cervical transformation zone usually by loop electrocautery (Cox et al., 2003). Chemopreventive therapies have, so far, been largely unsuccessful (Stanley, 2003).

HPV DNA testing is now approved by the U.S. Food and Drug Administration for the clarification of equivocal cytology ("ASC-US" Pap tests) at all ages, and as an adjunct to screening cervical cytology among women 30 and older (Wright and Schiffman, 2003). Because virtually all cases of cervical cancer are caused by oncogenic HPV, the absence of HPV as measured by sensitive reliable tests is very reassuring, especially at older ages when fewer new infections are expected (Franco, 2003).

Future Research

Although most future research will likely address the role of HPV in cervical cancer, but it will be important to understand the role of HPV in other cancers. Comparisons should be made of HPV natural history in different tissues. In particular, why are transformation zones so prone to HPV carcinogenesis?

It will be very important to compare oncogenic to low-risk types of HPV at the variant level in population-based studies, combining epidemiology and molecular biology. Given the small size of the HPV genome and our reasonable understanding of HPV carcinogenesis, it should be feasible to understand which parts of the viral genome influence risk of persistence and progression from infection to precancer/cancer given viral persistence. As a related issue, through continued prospective studies, the trend of decreasing anogenital HPV infection rates with increasing age should be better understood. Contributions of cohort effects, sexual practices, and immunologic suppression must be distinguished.

Secondary biomarkers indicating the interaction of host and virus are needed, particularly as diagnostic assays. Current HPV DNA detection methods, although sensitive, are inadequately specific to be the optimal answer for our testing needs because poor positive predictive value leads to excessive cost and iatrogenic morbidity (von Knebel, 2002; Wang and Hildesheim, 2003).

As the highest priority, HPV immunology is likely to occupy epidemiologists studying HPV infection over the next decade. In the immediate future, the interactions of multiple HPV types in mixed infections should be clarified, as one pathway to understanding HPV immunity. Assays of cell-mediated immunity must be developed and applied (Pinto et al., 2003). The association of specific HLA locus haplotypes with the risk of invasive cervical carcinoma might yield indirect evidence of the role of specific aspects of cell-mediated immunity in the natural history of HPV infections (Hildesheim and Wang, 2002). The ultimate goal will be to define the successful immune response to HPV infection, in the context of stimulating cancer-preventive immunity by vaccination.

RETROVIRUSES: HTLV-I

The human T-cell leukemia virus type I (HTLV-I) was the first identified human retrovirus. In 1980, Poiesz et al. made the original isolation from a cutaneous lymphoma in an African American. The same virus was isolated independently by Yoshida, Miyoshi, and Hinuma from a cell line established from a Japanese patient with T-cell leukemia (Yoshida et al., 1982). The malignancy, now called adult T-cell leukemia/lymphoma (ATL), an aggressive malignancy of mature T-lymphocytes, was first recognized as a distinct entity by Japanese
clinchs in the 1970s (Uchiyama et al., 1977). The syndrome is characterized by hypercalcemia, cutaneous involvement, and depressed cellular immunity (The T- and B-cell Malignancy Study Group, 1981). The evidence that the virus is a causal factor in the etiology of ATL is so compelling that it has been unchallenged: ATL being highly restricted to HTLV-I carriers has an estimated lifetime risk of ATL of ≤5% (IARC, 1996). Yet little is known about the natural history of the infection and the role of other factors that affect oncogenesis.

HTLV-II, a closely related retrovirus, has also been isolated (Kalyanaraman et al., 1982). This rare infection is primarily endemic among Ameindian groups but has reached a relatively high prevalence among American injection drug users (Liu et al., 2001). Although several early case reports linked HTLV-II infection with atypical hairy cell leukemia (Rosenblatt et al., 1986), this appears to be a very rare occurrence (Orland et al., 2003).

HTLV-I is a prototypic retrovirus most closely related to the bovine leukemia virus. Its genome of approximately 9 kb in length codes for a minimal set of structural genes plus several regulatory genes (Cann and Chen, 1996). The coding areas common to all retroviruses are gag (which encodes the core proteins p19, p24); env (envelope glycoproteins gp160, gp46); and pol, which encodes polymerase (reverse transcriptase), endonuclease (ribonuclease), and integrase. A protease is encoded by a reading frame spanning the gag and pol gene regions. The genome is bounded by long terminal repeats. It has an additional reading frame that codes for regulatory proteins, including tax (p40), rex (p27, p21), and several alternatively spliced mRNAs (p12, p13, p30). (The taxprotein was sometimes termed p42 in early publications.)

HTLV-I preferentially infects and transforms CD4+ T-cells, which express an activated phenotype, although CD8+ T-cells can also be infected (Cann and Chen, 1996). The oncogenic potential of HTLV-I in infected cells is largely orchestrated by the tax gene product (Johnson et al., 2001). The tax gene product promotes genetic mutation of infected cells, inhibits cell-cycle control, and drives viral gene expression. The tax protein constitutively activates the NFκB and SRF transcription pathways (Jeang, 2001). It activates key cellular genes, including IL-2, IL-2Rα, GM-CSF, and PTHRP, while down-regulating the p-polymerase and the cdk-inhibitory p16 genes. The fact that the integration sites of HTLV-I provirus differ between patients, and that no host oncoprotein has been identified within the provirus, argues that the mechanism of oncogenesis likely involves transactivation by tax (Seiki et al., 1983, 1984; Gatza et al., 2003).

Natural History of HTLV-I Infection

Unlike other retroviruses, such as FeLV or HIV-I, HTLV-I is a highly cell-associated infection, with predominantly cell-to-cell transmission and virtually no cell-free infectious virus produced in vitro (Igakura et al., 2003). This property accounts for its extremely low level of infectivity. Transmission primarily occurs by perinatal, blood, and sexual exposure. Perinatal infection principally occurs by prolonged breast feeding, presumably via infected lymphocytes. The transmission rate associated with lactation of ≥6 months is correlated with markers of high maternal proviral load; as such, it varies between groups but is generally about 20–30% (Hino et al., 1987; Hisada et al., 2002; Ando et al., 2003b). Interventions on breastfeeding of carrier mothers reduces, but does not eliminate, perinatal transmission (Ando et al., 2003a). The most efficient route of transmission is direct exposure to infected blood containing cellular components. In a prospective study of transmission by transfusion in Jamaica, 24 of 54 recipients of HTLV-I–positive, cellular blood components seroconverted while none of 12 recipients of positive, noncellular donor units, nor of 52 HTLV-I–negative units became infected (Manns et al., 1992). The screening of blood donors in endemic populations essentially eliminates this risk to transfusion recipients. However, the reuse of needles for injection and other applications, such as acupuncture, remains a potential source of infection. Sexual transmission is the most inefficient route, with infectivity of the exposing partner correlated with markers of high viral load. Transmission is more common from infected men to their female partners than the reverse (Shioiri et al., 1997).

In endemic populations, infection rates are quite low and stable among children, reflecting perinatal infection (Kusuhara et al., 1987). There is a slow increase of seroprevalence with advancing age, which plateaus among men at about age 50. Among older women, the seroprevalence continues to increase with age (Tajima et al., 1987; Mueller et al., 1990) (Fig. 26–7). Infection in adults appears to be due primarily to sexual exposure (Murphy et al., 1989; Stuver et al., 1993) and, to a much lesser extent, through transfusion. The divergence seroprevalence curves between genders after age 50 likely reflect the greater probability of heterosexual transmission from men than vice versa in this age group, but whether it is due to greater infectiousness of men, discontinuation of barrier contraception practices, or increased susceptibility following menopause is not known (Stuver et al., 1993).

Upon primary infection, HTLV-I provirus integrates into the genome of infected cells, establishing latency. As reviewed by Rosenblatt et al. (1988), the apparent reservoir of latent infection is in peripheral blood T-lymphocytes. These cells can be immortalized by HTLV-I in vitro, where they continue to grow in the presence of extraneous IL-2. The cells immortalized are generally of an activated T-helper cell phenotype, CD4+ and CD25+ (IL-2 receptor α), a prototype of essentially all cases of ATL.

It is thought that the oncogenic potential of HTLV-I infection is counterbalanced by host virus–specific CTLs, many of which are directed against the Tax protein (IARC, 1996; Bangham, 2000). (Of interest, HTLV-I–specific CTL response has been reported in seven of 19 seronegative/PCR negative persons who had known high exposure to the virus but in none of 16 matched controls without risk factors for exposure [Nishimura et al., 1994]). Although carriers make antibodies to various proteins of the virus, these antibodies do not appear to be protective once the infection is established. In fact, antibodies titers against structural proteins and against the Tax regulatory protein correlate with proviral load in asymptomatic carriers (Shioiri et al., 1993; Shinnzato et al., 1993; Ishihara et al., 1994; Morand-Joubert et al., 1995), suggesting they mirror viral protein expression. Zucker-Franklin et al. (1997, 1998) have reported asymptomatic antibody-negative individuals who are positive only for the tax gene sequences; however, this has not been confirmed (Cowan et al., 1999; Dezutti et al., 2003).

Carriers generally maintain their anti-HTLV-I antibody levels, suggesting that some level of viral replication occurs. However, the extremely low mutation rate of the virus at the population level—estimated at 0.1% per century (Gessain et al., 1992)—indicates that virus replication via error-prone reverse transcription is generally quite limited. Instead, a remarkable feature of HTLV-I infection is that viral

![Figure 26-7. Age-specific prevalence incidence of HTLV-I antibody by gender in Miyazaki Cohort (Japan) and Jamaican food handlers. (Source: Jamaican data adapted from Murphy et al., 1991.)](image-url)
expansion in asymptomatic carriers appears to occur predominantly by clonal expansion of infected cells (Furukawa et al., 1992; Wattel et al., 1995). We have shown that such clones can persist in asymptomatic Japanese carriers for years, apparently protected from host CTL response (Etoh et al., 1997). This remarkable biology is shared with the simian T-cell leukemia virus, which is phylogenetically intimately related to HTLV-1 (Vandamme et al., 1998), in its natural hosts (Gabet et al., 2003).

Paradoxically, it was reported by Mortreux et al. (2001) that somatic mutations do occur, both within the integrated provirus genomes and in flanking sequences, and accumulate proportionately with the prevalence of clones. The variation among clones could lead to selective evolution toward increased proliferation and potential malignancy. The authors reconcile the stability of the virus on the population level with the instability within infected clones of T-cells in vivo by proposing that the majority of somatically mutated proviruses are without virus progeny.

The clinical effects of primary HTLV-I infection are not characterized. The spectrum of HTLV-I antibodies evolves relatively quickly in seroconverters; however, the appearance of anti-Tax is delayed by a number of months (Manns et al., 1991). Antibody titer level and proviral load appear to stabilize within a few years after seroconversion (Okayama et al., 2001; Manns et al., 1999b). Of note, asymptomatic carriers show evidence of diminished cell-mediated immunity (Tachibana et al., 1988; Murai et al., 1990).

The disease outcomes of HTLV-I infection appear to be influenced by both age at infection and gender. Tajima and Hinuma (1984) first proposed that early age at infection may be a modifier of risk for ATL, as the sex ratio among ATL cases in Japan is essentially equal (with some male predominance) (Fig. 26-8), similar to the ratio of seroprevalence (Tachibana et al., 1988; Murai et al., 1990). The viral markers in HAM/TSP patients include high proviral load, elevated antibody titers against the virus and against Tax, and an elevated level of CTL against the Tax protein, consistent with its autoimmune nature (Nagai et al., 2001).

**Epidemiology of Infection**

The prevalence of HTLV-I infection is quite geographically restricted and exhibits a high level of clustering within endemic areas, consistent with interfamilial transmission (Stuver et al., 1992). Overall, it is estimated that there are 15–20 million HTLV-I carriers in the world. Areas with reported pockets of higher seroprevalence (>15% Vo) include the southwestern islands of Japan, the Caribbean, parts of South America, intertropical Africa, the Mashhad region in Iran, and Melanesia (IARC, 1996).

The epidemiology of HTLV-I infection presumably reflects the probable historic geographic distribution of infected populations, the limited transmissibility of the virus, and the corresponding low reproductive rate within populations. Phylogenetic analyses of viral isolates from endemic populations suggest that the virus is an ancient infection that originated in the Pacific Rim and spread by migrating populations throughout much of the world (Mansky, 2001). In phylogenetic trees, human isolates of HTLV-I interdigitate with simian counterparts, arguing that extensive interspecies transmission has occurred (Vandamme et al., 1998). There is extremely little variation between HTLV-I strains isolated from geographically diverse populations; for example, isolates from Japan, the Caribbean, and Africa share as much as 96–99% homology (Cann and Chen, 1996). There has been no demonstration of pathogenic differences between isolates from diverse endemic populations.

Most of the epidemiologic data that are available on HTLV-I come from Japan and the Caribbean where substantial research has been conducted and to a much lesser degree from Africa. Unfortunately, little is known about the epidemiology of HTLV-I–associated disease in carriers in the United States, given the rarity of the infection. American carriers tend to be descendants or immigrants from endemic populations (Ho et al., 1991; Parks et al., 1991; Kaplan et al., 1993; Dosik et al., 1994).

**HTLV-I Biomarkers**

Highly reliable assays are available and widely used in HTLV-I epidemiologic research. In the United States and Europe, HTLV-I antibodies are most commonly measured by EIAs, using whole virus lysate or recombinant antigens or peptides and in Japan, using anti-Tax antibodies are most commonly measured by EIAs, using whole virus lysate (Okayama et al., 1997). Antibody titer level and proviral load appear to stabilize within a few years after seroconversion (Okayama et al., 2001; Manns et al., 1999b). Of note, asymptomatic carriers show evidence of diminished cell-mediated immunity (Tachibana et al., 1988; Murai et al., 1990).

The viral markers in HAM/TSP patients include high proviral load, elevated antibody titers against the virus and against Tax, and an elevated level of CTL against the Tax protein, consistent with its autoimmune nature (Nagai et al., 2001).

**Adult T-Cell Leukemia/Lymphoma**

The strong geographic concordance between HTLV-I endemicity and the incidence of ATL in Japan provided the first major piece of evidence of a causal association. This concordance was carefully mapped by Japanese investigators in a series of four reports from the T- and B-cell Malignancy Study Group, published in the 1980s (Mueller, 1991). The clarity of the association was due to two factors: (1) the high level of geographic restriction and the micro-epidemiologic nature of HTLV-I infection, and (2) the vast majority of ATL in endemic populations occurs in HTLV-I carriers. The molecular evidence of the association was also consistent and persuasive. Antibodies against HTLV-I are detected in sera from nearly all AIL patients in endemic populations (Hinuma et al., 1981; Robert-Guroff et al., 1982). Assays to detect anti-Tax antibodies have been developed (Rudolph et al., 1994). In terms of indeterminate findings, cross-reactivity with Plasmodium falciparum has been reported (Hayes et al., 1991; Mahieux et al., 2000).

The direct assessment and quantification of proviral DNA is done using PCR techniques (IARC, 1996). Clonality of the virus can be assessed by Southern blot and by inverse PCR (Etoh et al., 1999). Reverse-transcription PCR can be used to detect mRNA to assess viral activity (Okayama et al., 1997).

**Figure 26-8. Age-specific incidence of ATL in Kyushu Prefecture, Japan, 1986–1987, by sex. (Source: Adapted from Tajima et al., 1990.)**

Legend:

- Females (grey line)
- Males (blue line)

- Y-axis: Incidence Rate per 100,000 persons per year
- X-axis: Age (in years)

- 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89, 90-100
The sequential elements in this model include an initial infection early in life, extensive clonal expansion of infected T-cells, the loss of Tax expression, and acquired genetic and epigenetic mutations. The apparent role of early infection suggests that an immature type 1 immunity at the time of infection increases the likelihood of developing a relatively high proviral load, perhaps compromising the subsequent immune maturation in such carriers. In proportion to high proviral load, an extraordinary number of clonal proliferations of HTLV-I-infected cells can occur over an extended period of time. The cellular proliferation and genetic instability that are orchestrated by Tax may become autonomous. By chance, the loss of Tax protein production in a proliferating clone then leads to a veil of protection from host CTL. One can envision the system spinning forward, stochastically inducing additional genetic and epigenetic changes that may result in malignancy.

The epidemiologic data based on the prospective development of ATL among HTLV-I carriers noted above confirm the hypothesis that higher proviral load is associated with the development of ATL. Of interest, the higher HTLV-I titers among persons who go on to develop ATL are not paralleled with antibody titers against the Tax protein, as is normally seen. Tax is not expressed on ATL cells, and following diagnosis, ATL cases exhibit a dissociation of anti-Tax antibody with HTLV-I antibodies (Yokota et al., 1989), likely reflecting the lack of active Tax protein expression. These two prospective studies confirm this finding and suggest that early in the pathogenesis of ATL, the premalignant clones become independent of ongoing Tax expression in the integrated HTLV-I provirus, thus escaping from CTL surveillance.

**Cofactors**

It has long been noted that HTLV-I-infected men are more likely to develop ATL than women, despite the higher infection rate among women (Kondo et al., 1987, 1989; Arisawa et al., 2000). The explanation for this difference may lie in gender differences in cell-mediated immune competency, which is central to the control of established HTLV-I infection. We have found that among carriers, men are about four times as likely to have diminished responsiveness to tuberculin skin testing—a functional measure of type 1 immunity (Hisada et al., 1999). This observation did not change with adjustment for estimated early age at infection (Hisada et al., 2001).

Among asymptomatic carriers, older men are more likely to have high proviral loads. Shinzato et al. (1991) reported that among 134 carriers from Nagasaki, those carriers with a high proviral load were all more than 30 years old and predominantly male. In the Miyasaki Cohort, a predominantly elderly population, we have found that carrier men are more likely to have a higher proviral load and detectable mRNA of the tax/rex gene than women (Tachibana et al., 1992; Okayama et al., 1997). That the effect of older, rather than younger age interacts with gender is underlined by two studies conducted among younger carriers. Etoh et al. (1999) found no difference between the genders for proviral load in a somewhat younger population of 256 Japanese carriers from Kumanoto. In a study of 20 younger asymptomatic carriers from the French West Indies, Wattel et al. (1992) also found no evidence of gender difference.

**Genetic Factors**

Genetic susceptibility to HTLV-I-induced malignancy has been considered. Several investigations have evaluated the HLA phenotypes of ATL patients; however, the findings have been inconsistent (Tajima et al., 1984; Tanaka et al., 1984; Usuku et al., 1988; The T- and B-Cell Malignancy Study Group, 1988). Usuku et al. (1988), Sonoda et al. (1993), and Yashiki et al. (2001) have defined two subgroups, as identified by HLA phenotype in Kagoshima who differ in their cellular immune response to HTLV-I in vitro. "Low responders" are associated with the occurrence of ATL, while "high responders" appear to be associated with risk of HAM/TSP. The distribution of HLA among normal carriers in the population tends to be intermediate.

**Strongyloidiasis**

It was initially proposed that risk of ATL was associated with undernutrition and with repeated exposure to filariasis in childhood, as both...
of these conditions were common in the past in endemic areas of Japan (Tajima and Hinuma, 1984). Neva et al. (1989) have reported no difference in the prevalence of antibody against *Strongyloides stercoralis* between HTLV-I carriers and controls in Jamaica. However, there is growing evidence that coinfection is detrimental to the HTLV-I carrier and associated with high proviral load. It appears that coinfected individuals have dysregulated immunity that diminishes their ability to control either infection. Porto et al. (2001) have reported that coinfected individuals’ type 2 immune responses that contribute to the control of the parasites, as measured by serum IgE and IL-5, are diminished. Conversely, Satoh et al. (2003) have found that coinfected individuals have a diminution of type 1 response, as indicated by decrease in anti-EBNA antibody levels for the EBV, with a corresponding increase in HTLV-I provirus load. Gabet et al. reported in 2000 that individuals with coinfection have five times higher proviral load, as compared to HTLV-I carriers who were not infected with S. stercoralis. Further, upon successful treatment for strongyloidiasis in one case, the HTLV-I proviral load dropped substantially and appeared to result from the extensive proliferation of a restricted number of infected clones. In contrast, in another case who had unsuccessful treatment for S. stercoralis, there was no significant effect on proviral load. Thus it appears that strongyloidiasis could enhance the risk of developing ATL in HTLV-I coinfected carriers.

**Social Environment**

Finally, it appears that the strongest factor that influences the risk of ATL within an endemic population is social and economic environment. This assertion is based on the evidence that the relative frequency and age at diagnosis of ATL and HAM/TSP differ markedly between Japanese carriers and those in the Caribbean. This finding does not appear to be due to differences in the virus per se in these two major population groups. Figure 26-9 displays the estimated age- and sex-specific incidence curves of ATL for Japanese and Jamaican carriers (Kondo et al., 1989; Murphy et al., 1989). In Japan, the incidence of ATL peaks at about age 60 for men; among women the incidence is about one-third that of men but also peaks among the elderly (Hisada et al., 2001). In sharp contrast, ATL occurs at a much lower rate in Jamaican carriers and peaks in the forties, with little evidence of a male predominance in incidence. For HAM/TSP, the preponderance of disease burden is reversed, with about a 10-fold higher incidence of HTLV-I-associated neurologic disorder seen in the Caribbean population. In both populations, women are at higher risk for HAM/TSP. In addition, a syndrome termed infectious dermatitis has been identified among perinatally infected Jamaican children (La Grenade et al., 1990). However, this condition has not been reported among infected Japanese children. Thus it appears that in the face of relatively optimum living conditions as in Japan, the effects of HTLV-I infection are less severe than in the face of poor living conditions, which are more prevalent in the Caribbean. In a comparative study of 51 age- and gender-matched HTLV-I carriers from Japan and Jamaica, we compared the viral markers for the two groups. We found that the mean antibody titer and detection of anti-Tax was significantly higher among the Jamaican carriers, while there was no significant difference in viral load (Hisada et al., 2004). The immunologic correlates with these population differences are currently under investigation.

**Prevention and Future Research**

The efficacy of prevention of transmission has been demonstrated for both breast milk and blood-borne exposure. Policies implementing these two interventions in endemic populations should bring the reproductive rate of HTLV-I infection below that needed to maintain endemic infection. Although the risk of sexually transmitted infection is low, it is nonetheless real, requiring sensible and sensitive counseling for discordantly infected couples.

In terms of epidemiologic research, what is essentially uncharted is the characterization of the immune profile of carriers in relation to viral markers; between genders, age groups, and host genetics; and between infected populations with differing disease outcomes. Such data should help flesh out the natural history of this infection and its variations by host and environment. To identify potential interventions in high risk carriers collaboration among the established cohorts is essential, given the rarity of prospective samples and data and the relatively low risk of disease among carriers. The most difficult route of transmission to interrupt is that of perinatal transmission in children who are not breastfed. This is a priority area that should not be neglected.

**HELICOBACTER PYLORI**

**Natural History of H. pylori Infection**

*Helicobacter pylori* is a Gram-negative, spiral-shaped organism that lives within the mucosa of the human stomach. The organism had been seen in histologic sections of the stomach since the early 20th century (Kreinitz, 1906), but not until the early 1980s was it recognized to cause disease—peptic ulcer disease, specifically. At that time, gastroenterologist Barry Marshall and colleagues in Perth, Australia, cultured the organism and also conducted epidemiologic studies connecting the organism to duodenal ulcer disease (Warren and Marshall, 1983; Marshall and Warren, 1984; Marshall et al., 1988). Subsequently, in two separate experiments, Marshall and Arthur Morris ingested *H. pylori* proving it caused the inflammatory process—gastritis with acute and chronic inflammation—that antecedes both ulcer disease and gastric adenocarcinoma (Walker et al., 1971; Marshall et al., 1985; Morris and Nicholson, 1987). From that time until today, a series of epidemiological studies has ensued to investigate the role of *H. pylori* in malignancy.

**Epidemiology of Infection**

*Helicobacter pylori* is an extremely common organism. It is estimated that 50% of the world is infected and, once infected, a person typically remains so for their lifetime (Brown, 2000). In the United States, Europe, and Oceania, the prevalence of *H. pylori* is probably closer to 25%, with infection quite unusual in children but continuing to plague the majority of adults over the age of 60 years. Over the last half of the 20th century in the industrialized West, *H. pylori* infection prevalence has decreased by a remarkable 25% per decade (Parsonnet et al., 1992; Banatvala et al., 1993; Roosendaal et al., 1997). This transformation parallels societal improvements 50 years earlier in socioeconomic status and in public health and hygiene. Infection is strongly linked with socioeconomic status in childhood (Malaty and Graham, 1994; Brown, 2000). In the United States, independent of
socioeconomic status, *H. pylori* is two- to threefold more common in blacks and in Hispanics than in whites (Everhart et al., 2000). One twin study suggests that there is a small element of genetic predisposition to the acquisition of infection (Malaty et al., 1994). Some studies also find *H. pylori* infection to be more common in men than in women, although this finding has not been consistently reported (Replogle et al., 1995; Everhart et al., 2000).

In developing countries, *H. pylori* infection is almost universally found in adults, and 50% of children may be infected by the age of 5 years (Brown, 2000). The difference in *H. pylori* prevalence in developing and developed countries is related to the mode of transmission. *H. pylori* is predominantly transmitted from person-to-person by the fecal–oral route. As with other organisms transmitted in this manner (e.g., hepatitis A and *Shigella*), improved sanitation and hygiene and decreased household crowding are accompanied by decreases in infection incidence. Because *H. pylori* infection, once established, does not spontaneously remit; the recent improvements in sanitation and hygiene lower childhood prevalence but have little effect on the prevalence in adults, who had acquired infection decades earlier.

All *H. pylori*-infected hosts develop chronic gastric inflammation or gastritis. This inflammatory process is associated with a specific humoral response as well as a locally vigorous Th1-mediated immune response, with elevated IL-12, IFN-γ, and TNF-α secretion (Crabtree, 1996; D’Elios et al., 1997). *H. pylori* also induces IL-8 secretion by epithelial cells, resulting in recruitment of inflammatory cells to the mucosa (Huang, 1995). In the chronic phase of infection, IL-10 has been reported to suppress the Th1 response (Holck et al., 2003). Some speculate that this IL-10 counter-response may foster bacterial persistence (Chen et al., 2001).

**Helicobacter pylori** Biomarkers

Large epidemiologic studies typically rely on serology (usually anti-*H. pylori* IgG) for diagnosis of infection. In older children and young and middle-aged adults who have not received treatment, serology has high sensitivity and specificity for ongoing infection (Vaira et al., 2002). In children less than 5 years, in the elderly, and those previously treated, however, *H. pylori* serology is less accurate. Moreover, not all tests work equally well in all populations (Bodhidatta et al., 1993). To be reliable, the tests should have been developed for a similar population to that in which the study is being conducted.

Serology is the only useful test for determining between types of *H. pylori* infection in large population-based studies. Specifically, *H. pylori* has two major subtypes: one that contains a pathogenicity island of genes called the Cag pathogenicity island (PAI) and one that does not. One of the Cag PAI proteins, CagA, is highly immunogenic, and antibodies to CagA are readily detected serologically. In CagA *H. pylori*-infected patients, antibodies to CagA are more sensitive for infection than the more general *H. pylori* serologic tests (Vaira et al., 2002).

Other genetic variants of *H. pylori*—including genotypes of the vacuolating cytotoxin (VacA), of a surface protein (BabA), and a protein expressed with bacterial adhesion to the mucosa (IceA) (van Doorn et al., 1998)—have been related to disease outcome. However, characterization of these variants requires endoscopy with bacterial culture, limiting their use in epidemiologic studies. Characterization by culture of the stomach is also relatively insensitive and potentially biasing, as not all strain types are equally cultivable ex vivo.

Other diagnostic tests for *H. pylori* include urea breath tests and stool antigen tests. These tests have sensitivity and specificity comparable to the best serologic assays (Vaira et al., 2002). The breath tests exploit *H. pylori*’s production of the enzyme urease—which splits urea into carbon dioxide and water—for diagnostic purposes. After giving an oral solution of carbon-isotope–labeled urea, investigators measure the amount of the isotope in exhaled carbon dioxide. Although C14 isotope tests are inexpensive, they are not commercially available in the United States. The less radioactive C13 tests are available, but their expense may limit their use in large epidemiologic studies. Stool antigen testing for *H. pylori* has not yet been used for many large studies although it is practically feasible.

**Associated Gastric Carcinoma**

Observational studies linking *H. pylori* with gastric adenocarcinoma include (1) ecologic studies correlating *H. pylori* prevalence and gastric cancer incidence and mortality in various geographic regions (Forman et al., 1990; Eurogast Study Group, 1993), (2) temporal studies demonstrating similar time trends in *H. pylori* prevalence and cancer incidence or mortality (Sonnenberg, 1993; Rupnow et al., 2000), (3) myriad case-control studies comparing *H. pylori* infection prevalence in gastric cancer patients and various controls (Huang et al., 1998; Eslick et al., 1999; Xue et al., 2001), (4) nested case-control studies (Helicobacter and Cancer Collaborative Group, 2001), and (5) one large cohort study demonstrating the temporal association between *H. pylori* infection and subsequent adenocarcinoma (Uemura et al., 2001). The resulting data paint a convincing picture that *H. pylori* is the preeminent risk factor for adenocarcinoma of the gastric antrum and the distal stomach. (Cancers of the proximal stomach, i.e., the gastroesophageal junction and cardia, are not thought to be *H. pylori*-related tumors.) In meta-analyses of the heterogeneous studies, case-control studies averaged a relatively low risk of cancer with infection (OR = 1.8); nested case-control studies found a higher risk (OR = 3.0); and nested case-control studies with long follow-up (>10 years) yielded an even higher estimate (OR = 5.9) (Xue et al., 2001; Helicobacter and Cancer Collaborative Group, 2001).

Although the nested case-control studies indicate that greater than 50% of gastric tumors are caused by *H. pylori*, gastric adenocarcinomas are not monomorphic. Different types of adenocarcinoma in the stomach have different risk factors. The types of gastric adenocarcinoma include the intestinal type (occurs more in men than in women, more in the elderly, and typically localize to the antrum), the diffuse type (seen equally in men and women, occur at younger age, and seen in antrum and body), and a much smaller number of lymphoepithelioma-like tumors (LELCs) (Lauren, 1965; Wang et al., 1999). *H. pylori* is a strong risk factor for both intestinal and diffuse tumors but not for LELCs (Huang et al., 1998; Wu MS et al., 2000).

Gastric tumors also differ by their location in the stomach. Tumors in the antrum predominate in developing countries, but this is not the case in industrialized countries. Currently, 50% of gastric adenocarcinomas in the United States are in the cardia or gastroesophageal junction (Devesa et al., 1998). *H. pylori* has been strongly linked to tumors in the antrum and body but less consistently linked to tumors of the cardia (Huang et al., 1998). Some data even support *H. pylori* being protective against cancers of the gastroesophageal junction (Chow et al., 1998). Given the variability of tumors and the imperfect nature of diagnostic tests, the true proportion of gastric tumors caused by *H. pylori* infection is difficult to state with certainty. However, some have speculated that *H. pylori* may be necessary for intestinal and diffuse tumors of the distal stomach to occur—the tumors most strongly linked to infection (Petersen, 2002).

Pivotal randomized clinical trials of *H. pylori* eradication in the prevention of gastric cancer are ongoing, although the logistics are daunting (Xia et al., 2003; Saito, 2003). Early analyses do not show that eradication of *H. pylori* prevents cancer per se, but rather evaluate whether *H. pylori* eradication prevents evolution or induces regression of the preneoplastic conditions that precede cancer. These data are inconclusive but suggest that *H. pylori* eradication may limit progression of preneoplasia to malignancy in a subset of subjects (Correa et al., 2000; Sung et al., 2000; Ley et al., 2004).

**Helicobacter pylori** has been even more strongly linked to primary gastric lymphoma than to adenocarcinoma (Boot and De Jong, 2002). Primary gastric lymphomas derive from marginal zone parafollicular B cells within mucosal-associated lymphoid tissue (MALT) (Ahmad et al., 2003). This tissue, which consists of submucosal lymphoid follicles that mimic the Peyer patches in the ileum, does not exist in the stomach of uninfected individuals but occurs in all with *H. pylori* infection. In a nested case-control study, infection was found in greater than 85% of primary gastric lymphoma, OR = 6.3 (Parsonnet et al., 1994). The true strength of the association between *H. pylori* and lym-
Mechanisms of Carcinogenesis

Chronic inflammation and augmented cell proliferation are believed to be critical factors in the development of gastric adenocarcinoma. The inflammatory process enhances production of free radicals by epithelial and inflammatory cells, leading to oxidative DNA damage (Baik et al., 1996; Farinati et al., 1998; Bagchi et al., 2002). Decreased antioxidant concentration (ascorbic acid, α-tocopherol, and β-carotene) in the gastric lumen and epithelium consequent to infection—which occurs irrespective of dietary antioxidants—enhances the probability of damage (Sobala et al., 1991; Zhang et al., 2000). Both through its DNA damaging effects as well as more directly through the action of its cytotoxin, H. pylori infection augments gastric epithelial apoptosis (Cover et al., 2003; Nagasaki et al., 2003). The mucosal epithelium manifests enhanced epithelial proliferation that may overcompensate for this apoptotic cell loss (Peek et al., 1997; Rokkas et al., 1999). In many, but not all infected hosts, this process leads to the gradual destruction of the mucosal glands (atrophic gastritis) and subsequent replacement of the normal tissue with metaplastic cells (intestinal metaplasia). Over time, glandular destruction results in decreased gastric acid secretory capacity, favoring extension of H. pylori’s domain throughout the stomach. Extensive, multifocal atrophic gastritis and intestinal metaplasia, although unnecessary for carcinogenesis, are considered preneoplastic conditions and markers for increased cancer risk.

Risk for cancer appears to be most acute with the strains that induce the most inflammation; specifically, those that contain the Cag PAI (Blaser et al., 1995; Parsonnet et al., 1997). Cag PAI strains typically coincide with a more virulent form of the vacuolating cytotoxin and are consequently more damaging to epithelial cells (Atherton et al., 1995). Additionally, the Cag PAI encodes a type IV secretion system that injects the CagA protein into epithelial cells (Stein et al., 2000). Within the epithelial cell, the CagA protein becomes phosphorylated and induces structural changes of the mucosal cell that give it a motile cellular phenotype. Individuals infected with strains containing Cag PAI have increased production of IL-1, TNF-α, IL-6, and IFN-γ when compared with infections of other strains (Helicobacter and Cancer Collaborative Group, 2001). Cag PAI strains also induce greater cellular IL-8 production and enhance inflammatory cell recruitment (Crabtree et al., 1995). These changes, in turn, result in greater free-radical production and augmented DNA damage. Cag PAI-containing strains increase the risk for intestinal type tumors—which often contain the classic CpG mutations seen with inflammation-related malignancies—threefold compared to non-Cag PAI strains; however, Cag PAI does not appear to engender greater risk for diffuse tumors than other strains (Parsonnet et al., 1997; Shibata et al., 2002). Thus, the mechanisms of H. pylori-related disease may vary for different tumors and different infections.

The pathogenesis of MALT, which comprises up to 50% of gastric lymphomas (Isaacsan et al., 2001), is poorly understood. It appears H. pylori infection stimulates T cells to drive B-cell proliferation in MALT (Hussell et al., 1993). The early stage of MALT lymphoma with low-grade histology and involvement of only superficial areas of the mucosa is completely reversible with H. pylori eradication therapy. On careful examination of lymphocytes remaining in the tissue, however, clonal cells from the lymphoma may still be found despite regression, and recurrent infection can result in recurrent tumor. Eventually, if untreated, a translocation t(11;18)(q21;q21) causes the B-cell proliferation to no longer respond to H. pylori eradication (Boot and De Jong, 2002).

Cofactors

Only a small proportion of H. pylori–infected people develop gastric adenocarcinoma. Factors that contribute to the outcome in this small subset include bacterial factors (described above), host factors, environmental cofactors, and age of acquisition of infection.

To date, the strongest host factors for cancer related to infection appears to be the IL-1β genotype and the genotype of the IL-1β endogenous receptor antagonist (El Omar et al., 2000). The disadvantageous IL-1β genotype both enhances the inflammatory response and decreases acid secretion within the stomach. Similar links to polymorphisms of TNF-α and IL-10 have also been described (El Omar et al., 2003). Combinations of these adverse cytokines markedly enhance risk, whereas none of these cytokine variations is related to cancer in uninfected hosts.

Environmental cofactors are poorly understood. Among the most intriguing of the putative cofactors are coinfections with other infectious agents. Some maintain that decreased gastric acidity enhances H. pylori–related carcinogenesis by permitting bacterial growth of N-nitrosating organisms within the stomach (Correa et al., 1975; Sanduleanu et al., 2001). Based on a mouse model, others have postulated that intestinal helminths mitigate the probability of cancer in the setting of H. pylori infection by diminishing the mucosal inflammatory response (Fox et al., 2000). Noninfectious factors that may alter cancer risk in the setting of H. pylori include dietary antioxidants, aspirin, and smoking (Ekstrom et al., 2000; Siman et al., 2001; Akre et al., 2001). Each of these hypotheses requires further substantiation.

As with hepatitis B infection, age at acquisition of infection may contribute to infection outcome. Because the age at which infection is acquired is typically unknown, this is a difficult hypothesis to test. Some circumstantial data, however, support this possibility. First, H. pylori–infected first-born children, who typically have less exposure to oral–fetal pathogens than children later in the birth order, are less likely to get gastric cancer than others (Blaser et al., 1994). This suggests that later acquisition of infection may be protective. Second, fetal–oral pathogens that are on the wane (hepatitis A and Shigella) exhibit a characteristic change in age of incidence. Specifically, as the incidence of infection decreases, the average age at infection acquisition increases. A pattern of decreasing overall incidence of infection, increasing age of acquisition, and decreased average duration of infection does much to explain patterns of cancer and ulcer disease observed worldwide.

Cofactors for lymphomagenesis are unknown. The PAI status of H. pylori infection does not appear to influence the occurrence of MALT lymphoma.

Prevention

Helicobacter pylori is spontaneously disappearing from Western populations (Parsonnet et al., 1992; Banatvala et al., 1993; Roosendaal et al., 1997). It is increasingly rare to find infection in children born in the United States, Western Europe, Japan, or Australia. Primary prevention of infection may therefore be moot in the industrialized West. Improvements in household sanitation and hygiene, decreased household crowding, provision of clean water, and improved nutrition coincide with the organism’s demise. Exactly which of these factors is most responsible for H. pylori’s disappearance is unknown. Based on its primary mode of transmission—person-to-person spread—it is most likely that the combination of household sanitation and hygiene and decreased crowding have mitigated the incidence of infection. With that, analyses indicate that an H. pylori vaccine could be a cost-effective strategy for cancer prevention (Ruppow et al., 1999; 2001). The years of use of such a vaccine would be limited in developed countries, as the organism would rapidly spiral out of existence. In developing countries and countries undergoing rapid economic growth where the incidence of H. pylori remains high and gastric cancer is common, a vaccine could be useful for the foreseeable future. The challenges of creating such a vaccine, however, are daunting. H. pylori is a chronic infection that can evade the human immune response for...
decades. Some even posit that the natural host response benefits the organism. Yet, experimental and accidental ingestions of the organism indicate that some people do not get infected despite exposure. Much more work needs to be done to understand the correlates of this protective immunity.

Secondary prevention is widely available. *H. pylori* is curable with combinations of antibiotic therapy. Although increased resistance to treatment is being observed, the diligent clinician can eradicate virtually any infection. Controversy remains over who should receive such treatment. A majority of the world’s population is infected with *H. pylori*, yet only a small percent will develop malignancy. Although this small ratio of benefited-to-treated challenges the probability of success, several analyses indicate that eradication of *H. pylori* could be cost-effective in preventing cancer (Parsonnet et al., 1996; Fendrick et al., 1999; Mason et al., 2002). To be cost-effective, however, such treatment would need to be given in midlife (Parsonnet et al., 1996).

Unfortunately, it is unknown whether treatment of infection so late in its course diminishes cancer incidence. Studies on *H. pylori* preneoplastic conditions have not promised a large benefit in adults. It is hoped that randomized trials addressing cancer prevention more directly will definitively provide answers to this question.

Until such studies are complete, different consensus conferences have advocated different strategies. The NIH Consensus, the oldest of the available recommendations, has not advocated treatment of asymptomatic patients to prevent cancer (NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease, 1994). In contrast, the more recent European Consensus has recommended treatment for people with a family history of gastric cancer, people who have had partial gastrectomy, those with advanced preneoplasia on gastric biopsy, and for virtually any one who desires treatment (Malfertheiner et al., 2002).

Tertiary prevention (i.e., treatment of *H. pylori* after diagnosis of adenocarcinoma) is widely undertaken in patients who have early, potentially curable malignancies. A small, nonrandomized trial in Japan found that patients with mucosally resected early gastric cancer did not have disease recurrence after *H. pylori* eradication therapy; without *H. pylori* eradication, recurrence occurred at the rate of 10% per year (Uemura et al., 1997). Treatment of *H. pylori* is also widely recommended for patients with MALT lymphoma, where in early stages, *H. pylori* eradication may be curative.

**Future Research**

The most critical area in the *H. pylori*-cancer field is to see whether, and when, *H. pylori* eradication prevents cancer. Can treatment reverse disease progression in midlife? At what age, if any, are these treatment benefits most evident? Are there specific strains that should be treated and others ignored? If so, how can this best be done? A second area of intense debate is whether deficits of *H. pylori* eradication outweigh benefits. Several studies indicate that *H. pylori* eradication therapy increases the risk of esophageal reflux disease (Falk, 2001) and possibly that of esophageal cancer (Henrik et al., 2001). The latter is among the most rapidly increasing tumors in the world. In favor of *H. pylori* eradication, the organism also causes ulcer disease and, like other chronic inflammatory processes, is suspected of contributing to atherogenesis. What is the balance of all of these conditions? Randomized trials of therapy that simply address *H. pylori*'s role in gastric cancer may not be enough to settle the debate regarding secondary prevention. We need to evaluate a variety of outcomes, including total mortality. Moreover, because strains of varying pathogenicity exist and have differing distributions worldwide, decisions to screen and treat may need to be made not broadly but regionally, based on local data regarding *H. pylori* genotypes, cofactors for disease, and the patterns of cancer risk.

*H. pylori* infection is one of the strongest risk factors for cancer identified. Identifying its importance has been a far simpler endeavor than deciding how to address the problem. This debate will undoubtedly continue for the next decade. Yet it is to be hoped that we will ultimately have solid solutions for treatment and prevention of this curable carcinogen.

**PARASITES**

**Schistosomes**

Schistosomes form a genus of parasitic blood flukes, three of which have been evaluated with respect to their carcinogenicity to humans (IARC, 1994b): *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*. *Schistosoma mansoni* and *S. haematobium* account for more than 95% of human infections and are endemic in Africa and the eastern Mediterranean; *S. mansoni* is also found in Central and South America. *Schistosoma japonicum* occurs primarily in Southeast Asia, including China, the Philippines, and Indonesia. Infection results from exposure to contaminated freshwater during which the schistosomal larvae penetrate the skin. These larvae mature and travel through the bloodstream to the veins that drain the urinary bladder (*S. haematobium*) or the gastrointestinal tract (*S. japonicum* and *S. mansoni*), where they produce a large number of eggs. The eggs are then excreted in the host’s urine or feces, respectively. Many eggs also remain within the tissue of the bladder and ureters (*S. haematobium*) or the intestines and liver (*S. japonicum* and *S. mansoni*). The larvae hatched from eggs that are shed into freshwater then infect an intermediate snail host within which they multiply by an asexual process and are released back into the water.

Detection of infection is based on the observation of eggs in urine (*S. haematobium*) or feces (*S. japonicum* and *S. mansoni*) (IARC, 1994b). Assays to detect parasite-specific antibodies or antigens appear to be of variable validity and have not generally been used in epidemiologic studies. The prevalence and intensity (eggs per milliliter of urine or per gram of feces) of infection increases from childhood through about age 20 and then declines. Manifestation of clinically apparent schistosomiasis results from the host immune response to the parasite eggs and the subsequent development of granulomas in the affected organs. The extent of associated morbidity appears to be related to the intensity and duration of the schistosomal infection, although many infected persons do not experience any significant symptoms. The adult worm can live in a human host for up to 30 years.

A fairly large number of case series studies conducted in Africa have linked the presence of *S. haematobium* infection with bladder cancer (IARC, 1994b). Such schistosomal-related tumors are generally squamous cell in origin and occur at a relatively young age, in contrast to other bladder cancers. Correlation studies, again performed primarily in Africa, also support an association between *S. haematobium* endemcity and bladder cancer occurrence (Thomas et al., 1990; IARC, 1994b). Statistically significant RRs ranging from 2 to 15 have been found in about seven hospital-based case-control studies (IARC, 1994b; Vizcaino et al., 1994; Bedwani et al., 1998). Based on these data, *S. haematobium* has been classified by IARC as being carcinogenic to humans.

With respect to the malignant potential of infection with *S. japonicum*, rates of colorectal and of liver cancer have shown significant geographic correlations with the impact of this parasite within Japan and China (IARC, 1994b). In the IARC assessment of the association of *S. japonicum* with cancer development, only three case-control studies for each of liver cancer and colorectal cancer provided relevant data. Although significant RRs were reported by these studies, of the order 2 to 10, the limited available evidence led IARC to conclude that infection with *S. japonicum* is “possibly carcinogenic” (Class 2B). Inadequate information existed to evaluate *S. mansoni* (IARC, 1994b).

The hypothesized mechanism by which chronic *S. haematobium* infection induces bladder cancer is via immune-mediated inflammation of the tissues in which the parasite eggs are embedded (IARC, 1994b). The resultant cell turnover and regeneration likely leads to the promotion of existing mutations, with concomitant proliferation of and dysplastic changes in the squamous epithelium of the bladder and lower urinary tract. Moreover, during the inflammatory process, mutagenesis may occur from the production of carcinogenic metabolites as well as nitrosonitrogen compounds.

An estimated 9500 cases of bladder cancer occurring in 1990 were attributable to infection with *S. haematobium* (Parkin et al., 1999). All of these cases were located in Africa or West Asia and represented
one-third of the bladder cancers in those areas. Schistosomal infection can be effectively treated. Although costly, population-based chemotherapeutic interventions, combined with public health efforts to improve sanitation, safeguard water supplies, and increase health education at the community level, have led to substantial decreases in, and even eradication of, the burden of schistosomiasis in Asia, Latin America, major parts of the Middle East and the Caribbean, and other endemic areas (IARC, 1994b; WHO, 2002). However, the prevalence of schistosomes and the morbidity associated with these infections remain elevated in sub-Saharan Africa.

Liver Flukes

Infection with three species of liver flukes has been hypothesized to be associated with cholangiocarcinoma of the liver: Opisthochiris viverrini, Opisthochiris felineus, and Clonorchis sinensis. These parasites can be found at highly endemic levels in Thailand and Laos (O. viverrini), China, Taiwan, and the Republic of Korea (C. sinensis), and the Russian Federation (O. felineus) (IARC, 1994b). Transmission to humans occurs via the consumption of raw fish contaminated with the infectious miracid, a stage of the liver fluke life cycle. Upon hatching, the flukes migrate to the bile ducts where they mature and lay eggs that are shed in the feces. Subsequent fecal contamination of freshwater leads to the ingestion of the eggs by susceptible snails, which serve as the intermediate host for the reproductive stage. Larvae are then released and infect fish, which are the second intermediate host in the liver fluke’s life cycle.

Infection with liver flukes is most usually determined by the detection of eggs in fecal specimens (IARC, 1994b). ELISAs also have been used to measure helminth-specific antibodies. In endemic areas, the prevalence of infection is relatively low in young children, generally increases during the early to late teen years, and then plateaus (IARC, 1994b). Liver flukes establish a chronic infection, unless treatment is received, and can live up to 25 years in the infected human host.

The epidemiologic evidence linking liver fluke infection with the occurrence of cholangiocarcinoma has originated from correlation studies and a small number of case-control studies. Much of the research has been conducted in Thailand and, thus, pertains to infection with O. viverrini (Parkin et al., 1993; IARC, 1994b). In that country, a strong correlation has been observed between cholangiocarcinoma rates and the prevalence of O. viverrini (Srivatnakul et al., 1991). A hospital-based case-control study reported a fivefold association between incident cholangiocarcinoma and elevated antibodies to this parasite (Parkin et al., 1991). In addition, in a cross-sectional study conducted in northeastern Thailand, an increasing odds of prevalent cholangiocarcinoma, as detected by ultrasonography, was found for increasing intensity of O. viverrini infection (Haswell-Elkins, 1994a). The data related to a carcinogenic effect of the other two liver fluke infections are more limited, particularly for O. felineus (IARC, 1994b). Case series studies of cholangiocarcinoma and bile duct cancers occurring in Hong Kong and Korea demonstrated a likely relationship with C. sinensis infection. One hospital-based case-control study in Hong Kong and two in Korea observed RRs of 3 and 6, respectively, for O. viverrini infection (Parkin et al., 1993; IARC, 1994b). IARC has categorized infection with O. viverrini as being Class 1 human carcinogen as infection with C. sinensis as being “probably carcinogenic” (Class 2A) (IARC, 1994b).

Chronic infection with liver flukes is believed to induce carcinogenesis through the mechanism of chronic inflammation of the intrahepatic bile ducts, resulting from direct irritation of epithelial cells by the liver flukes as well as from the host immune response to the parasites (Parkin et al., 1993; IARC, 1994b). The carcinogenic process is likely similar to that for schistosomal infection, with inflammation-associated changes playing an important role. Additional mutations also may be produced by the generation of reactive oxygen species (Parkin et al., 1993). As well, the induction of nitric oxides and nitrosamines may be particularly relevant with respect to the development of parasite-related cholangiocarcinoma (Haswell-Elkins, 1994b).

Despite the high prevalence of these infections in endemic areas of Southeast Asia and Russia, cholangiocarcinoma is relatively rare in affected populations. The number of cases attributable to O. viverrini infection in Thailand in 1990 was estimated to be about 300, although perhaps as high as 3000 (Parkin et al., 1999). Effective treatment is available for liver fluke infection, which would prevent further damage to the biliary tract and progression to cancer. Improvements in sanitation and education with respect to the consumption of raw fish also are important for reducing the burden of infection in endemic areas.

FUTURE DIRECTIONS

Despite their diversity, these persistent infections share some common features in their demonstrated oncogenicity. These include the central importance of the dynamic host-agent interaction. Age and other host conditions of initial infection as well as gender are often important in influencing the host-agent interaction. The direct modification of cell-cycle control is a characteristic of most of these infections. Malignancy occurs as an occasional accident.

The empirical use of agent-related biomarkers has illuminated the trail for untangling the epidemiology of these systems. A key area for future research in all of the oncogenic infections is the characterization of immune status in biospecimens gathered during the formative period of malignancy. An early model of such research is that of Schroeder et al. (1999), who demonstrated the predictive value of serum sCD23 for subsequent non-Hodgkin lymphoma in AIDS patients. The identification of the determinants of cytokine profile, including such factors as gender, social factors, and genetic polymorphisms, should fill in the evolving “pictures” of the natural history of these infection-associated malignancies.

In terms of secondary prevention, the tools are in hand to help identify those at high risk in most of these infection-associated cancers. In fact, we have an example of infection-associated malignancy that can be cured by treatment of the underlying infection in MALT and an example of reversal of a premalignant disease in post-transplant lymphoproliferation with the infusion of EBV-specific CTLs. It is likely that interventions on the other oncogenic infections to prevent or reverse oncogenesis will be seen.

New associations between infections and cancer continue to be proposed and can generate contentious scientific interest and great public concern. The prime current example is the question of whether SV40 is a human carcinogen (Klein et al., 2002). We propose that the validity of such assertions be addressed quickly and rigorously and be guided by the body of work summarized here. However, we should be open to new twists in this engaging set of puzzles.

References


Infectious Agents


Infectious Agents


Silverberg MJ, Thrason P, Lindeberg H, Grant LA, Shah KV. 2003. Condyloma in pregnancy is strongly predictive of juvenile-onset recurrent respiratory papil-

Siman JH, Forsgren A, Berglund G, Floren CH. 2001. Tobacco smoking increases the risk for gastric cancer among Helicobacter pylori-infected individ-


53–70.


mediated diseases driven by Th1 and Th2 subsets suggests a common aeti-


Sirianun U, Leitmann L, Iregang B, Dussaith C, Bauchart J, Bataille V. 1996. Human her-

pesvirus 8 latent infection and is restored upon highly active antiretroviral therapy in AIDS patients with regressing Karposi's sarcoma. Eur J Immunol 32:2711–2720.

Sitas F, Newton R, Bosshoff C. 1999. Increasing probability of mother-to-child trans-


letin 5:79–88.


2417.


Stein M, Rappuoli R, Covacci A. 2000. Tyrosine phosphorylation of the Hel-


Tanaka K, Ikematsu H, Hirohata I Kashiwagi S. 1996. Hepatitis C virus infection
Tabor E. 2003. Interferon for preventing and treating hepatocellular carcinoma asso-
Swinnen LI, Gulley ML, Hamilton E, Schichman S. 1998. EBV DNA quanrirarion
Tellof PE, Brown D, Devereux H, Lee CA, Desheki GM. 1994. HCV RNA levels and
Terrault NA. 2002. Sexual activity as a risk factor for hepatitis C. Hepatology
Thio CL, Seaberg EC, Skolasky R, et al. 2002. HIV-1, hepatitis B virus, and risk of
Vall Mayans M, Calvet X, Bruix J, et al. 1990. Risk factors for hepatocellular car-
Tappero JW, Conant MA, Wolfe SF, Berger TG. 1993. Kaposi’s sarcoma: Epidemi-
Takahashi M, Yamada G, Miyamoto R, Doi T, Endo H, Tuji T. 1993. Natural course of
PART III: THE CAUSES OF CANCER


