GENERAL REMARKS

1. Background

This one-hundred-and-fourth volume of the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* is the eighth volume devoted to infectious biological agents. The biological agents that have been considered to date by the *IARC Monographs* are listed in Table 1. Several have been recognized as major risk factors involved in the burden of cancer.

In Volume 104, the *IARC Monographs* consider the following agents for the first time: malaria (a disease caused by infection with the *Plasmodium* parasite) and four polyomaviruses: the simian virus SV40, and the BK, JC, and Merkel cell polyomaviruses. Infection by these microorganisms concerns a very large proportion of the world population.

A summary of the findings of this volume appears in *The Lancet Oncology* (Bouvard *et al.*, 2012).

2. Malaria

The global burden of malaria is enormous; about 50% of the world's population is at risk of developing malaria, with an estimated annual incidence of 219 million cases worldwide (range, 154–289 million), mostly in sub-Saharan Africa and Papua New Guinea (WHO, 2011). A role for malaria in the etiology of endemic Burkitt lymphoma in areas where malaria is highly endemic (holoendemic) has long been suspected (Burkitt, 1961). In these areas, this type of non-Hodgkin lymphoma can account for up to 70% of childhood cancers, with the highest incidence at age 5–9 years (Parkin *et al.*, 2008).

While malaria is caused by several species of *Plasmodium*, the majority of studies investigating the link between malaria and cancer have been conducted in areas where *P. falciparum* is highly prevalent, or have focused deliberately on *P. falciparum* (the species linked to the most severe form of malaria).

2.1 Ecological studies

Major epidemiological studies of malaria (infection with *P. falciparum)* and cancer, mainly Burkitt lymphoma, include ecological studies and fewer case–control and cohort studies. For most carcinogens, causal inference is primarily based on case–control and cohort studies, with little, if any, support from ecological studies. For malaria, in contrast, ecological studies provide important

Table 1 Evaluation of the carcinogenicity to humans of biological agents from this and previous *IARC Monographs* volumes

Biological agent	IARC Group	*IARC Monographs* volume (year)
Hepatitis B virus	1	Vol 59 (IARC, 1994a); Vol 100B (IARC, 2012)
Hepatitis C virus	1	Vol 59 (IARC, 1994a); Vol 100B (IARC, 2012)
Hepatitis D virus	3	Vol 59 (IARC, 1994a)
HIV-1	1	Vol 67 (IARC, 1996); Vol 100B (IARC, 2012)
HIV-2	2B	Vol 67 (IARC, 1996)
HTLV-I	1	Vol 67 (IARC, 1996); Vol 100B (IARC, 2012)
HTLV-II	3	Vol 67 (IARC, 1996)
EBV	1	Vol 70 (IARC, 1997); Vol 100B (IARC, 2012)
KSHV	1	Vol 70 (IARC, 1997); Vol 100B (IARC, 2012)
HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59	1	Vol 64 (IARC, 1995); Vol 90 (IARC, 2007); Vol 100B (IARC, 2012)
HPV type 68	2A	Vol 64 (IARC, 1995); Vol 90 (IARC, 2007); Vol 100B (IARC, 2012)
HPV types 26, 30, 34, 53, 66, 67,69, 70, 73, 82, 85 and 97	2B	Vol 64 (IARC, 1995); Vol 90 (IARC, 2007); Vol 100B (IARC, 2012)
HPV types 6 and 11	3	Vol 64 (IARC, 1995); Vol 90 (IARC, 2007); Vol 100B (IARC, 2012)
Some HPV of genera beta and gamma	3	Vol 64 (IARC, 1995); Vol 90 (IARC, 2007); Vol 100B (IARC, 2012)
HPV types 5 and 8 of genera beta	2B	Vol 64 (IARC, 1995); Vol 90 (IARC, 2007); Vol 100B (IARC, 2012)
SV40 simian polyomavirus	3	Vol 104 (this volume)
BK polyomavirus (BKV)	2B	Vol 104 (this volume)
JC polyomavirus (JCV)	2B	Vol 104 (this volume)
Merkel cell polyomavirus (MCV)	2A	Vol 104 (this volume)
Malaria (infection by *Plasmodium falciparum* in holoendemic areas)	2A	Vol 104 (this volume)
Schistosoma haematobium	1	Vol 61 (IARC, 1994b); Vol 100B (IARC, 2012)
Schistosoma mansoni	3	Vol 61 (IARC, 1994b)
Schistosoma japonicum	2B	Vol 61 (IARC, 1994b)
Helicobacter pylori	1	Vol 61 (IARC, 1994b); Vol 100B (IARC, 2012)
Opistorchis viverrini	1	Vol 61 (IARC, 1994b); Vol 100B (IARC, 2012)
Opistorchis felineus	3	Vol 61 (IARC, 1994b)
Chlonorchis sinensis	1	Vol 61 (IARC, 1994b); Vol 100B (IARC, 2012)

EBV, Epstein–Barr virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HTLV, human T-lymphotropic virus; KSHV, Kaposi sarcoma-associated herpesvirus

information on causal inference because of large differences in exposure in areas with different burdens of infection with *P. falciparum* (particularly in children) and limited population migration. Particularly in areas of holoendemic malaria, ecological measures provide a strong indication of individual exposure. However, the Working Group found that there were often limitations in the assessment of exposure in pertinent analytical studies (see below). Hence, in the *Monograph* on malaria, ecological studies were described in detail and assessed with consideration of the complexity of the parameters that influence the global epidemiology of malaria.

The burden of infection with *P. falciparum* within a population is described ecologically on the basis of transmission intensity. The definition of transmission intensity for falciparum malaria is based on exposure in children aged 2–10 years and measured by entomological inoculation rate (EIR), parasite reproductive numbers, prevalence of parasites in peripheral blood, and frequency of splenomegaly. Holoendemic malaria transmission has been defined as parasite prevalence of > 70% and splenomegaly in > 80% of children.

2.1.1 Environmental ecology, vector-species identification and locally dominant parasites

Malaria is a mosquito-borne infection and so the biology of this disease is closely linked to the environmental ecology of the species of *Anopheles* mosquito responsible for transmission of the parasite. This explains the geographical distribution of malaria, the differences in transmission intensity that are mostly dependent on climatic variables (e.g. altitude, season), and the differences seen in rural versus urban populations.

2.1.2 Diagnosis of malaria

The correct diagnosis of malaria remains a challenge in many countries. The burden of malaria can be overestimated when diagnosis is based on symptoms alone, since the symptoms typical of malaria (e.g. fever, chills) are also exhibited during other microbial infections (Ari *et al.*, 2011). In sub-Saharan countries, the burden of infectious disease is high, and co-infection by *P. falciparum* and a second infectious agent (e.g. hookworm or *Mansonella perstans)* in the same individual is common (Hillier *et al.*, 2008). In contrast, reliance on passive national reporting of malaria has likely led to underestimation of the true burden of malaria worldwide (Snow *et al.*, 2005).

2.1.3 Variation in host genetic susceptibility

Genetic polymorphisms known to influence the severity of malaria (e.g. sickle-cell trait) are widespread in African people and may also have a role in susceptibility to cancer.

2.1.4 Modifiable factors

Modifiable factors such as intense use of chemically treated bednets, use of indoor spraying with insecticides, and availability of effective antimalarial therapy may also have a strong influence on the global burden of malaria.

2.2 Analytical epidemiological studies

Only one cohort study and few case–control studies on the link between malaria and Burkitt lymphoma have been reported, and even fewer have looked at other cancers (e.g. Kaposi sarcoma). For these studies, uncertainty about the accuracy of the methods used for the assessment of exposure to *P. falciparum* infection was a major limitation. Although the use of serology for the detection of antimalaria antibodies may appear to be a better means of exposure assessment than questionnaires, accuracy of the results is highly dependent on the choice of antibodies used as markers of infection. It has been shown that immunoglobulin G (IgG)-specific antimalaria antibodies are often short-lived in young children and levels only increase with age and repeated infections. Therefore, use of such antibodies tends to underestimate past infection with *P. falciparum*. The choice of malaria antigen against which such antibodies are targeted can also be problematic because of the well documented genetic variation in the surface antigens of *P. falciparum*. Measurement of antibodies to antigens derived from whole schizont extracts is thought to provide a good estimate of past exposure to *P. falciparum* (Marsh & Kinyanjui, 2006).

2.3 Burkitt lymphoma, Epstein–Barr virus and malaria

Epstein-Barr virus (EBV) has been established as an essential etiological agent for endemic Burkitt lymphoma (IARC, 1997, 2012). More than 90% of the population worldwide is infected with this highly oncogenic virus. However, infection is asymptomatic in most carriers since the virus usually remains latent. Most studies on Burkitt lymphoma have looked at the role of EBV reactivation per se, without looking at the potential effect of malaria status; likewise, most studies reporting on the association between malaria and Burkitt lymphoma did not consider EBV status. Based on two recent case–control studies looking at the effects of co-infection with EBV and *P. falciparum*, and on strong mechanistic data showing clear interactions between EBV and *P. falciparum*, the Working Group concluded that endemic Burkitt lymphoma is likely to be caused by the combined effects of early co-infection with EBV and malaria in a holoendemic area.

3. Polyomaviruses

In the 1950s and early 1960s, millions of people worldwide received poliovirus vaccines that were contaminated with SV40, a polyomavirus that naturally infects the rhesus macaque. Shortly after the discovery of this contamination, SV40 was shown to induce tumours in newborn hamsters; there have thus been serious concerns that SV40 may cause cancer in humans who received the contaminated virus.

In 1971, BK polyomavirus (BKV) and JC polyomavirus (JCV) became the first naturally human-tropic polyomaviruses to be isolated. Merkel cell polyomavirus (MCV) was discovered more recently, in 2008, in a rare skin cancer, Merkel cell carcinoma. Infection with human polyomaviruses is widespread in the general population, with about 50% to > 90% of adults being infected worldwide.

During the evaluation process, the Working Group raised several issues inherent to the characteristics of the polyomaviruses evaluated in this volume and that are important to consider when interpreting the data. These issues are summarized below.

3.1 Specificity of the detection methods

In epidemiological studies, detection of infection by polyomaviruses has been mostly based on the amplification of viral DNA by polymerase chain reaction (PCR), and past infections have been assessed by the detection of antibodies raised against the viral capsid. Concerns have been raised regarding both types of method.

3.1.1 PCR-based methods

PCR techniques are extremely sensitive; laboratory contamination, lack of specificity or cross-amplification with other polyomaviruses might explain some discrepancies observed between studies.

BKV and JCV are very closely related to one another and to SV40; the three viruses share about 70–75% identity at the nucleotide level across the entire genome. Indeed, primers initially designed to amplify SV40 DNA sequences can cross-amplify BKV or JCV sequences. Furthermore, some PCR primers used have been shown to co-amplify contaminating SV40 sequences present in common laboratory plasmids and in some laboratory cell lines. Consequently, and also because of the ubiquitous presence of BKV and JCV viruses in the human population, it was necessary to apply caution in the interpretation of the PCR results e.g. false-positive results in studies assessing the presence of SV40 in human cancers.

3.1.2 Serological methods

Detection of antibodies to polyomaviruses in human sera is performed mostly by neutralization tests or enzyme-linked immunosorbent assays (ELISA) using recombinant virus-like particles (VLPs). Because the structural organization of some polyomaviruses is highly conserved, competitive-inhibition studies are necessary to ensure the specificity of the antibodies detected. Indeed, it has been suggested that reactivity to SV40 in human sera reported in some studies is due to cross-reactivity to BKV and JCV, since reactivity to SV40 is often eliminated by pre-incubation with BKV or JCV VLPs.

Assays for haemaglutination inhibition (HAI) have also been used to measure titres of antibodies to BKV in human sera, since BKV cause agglutination of human erythrocytes of group O; however, since JCV has also now been shown to be capable of haemaglutination, HAI is not completely specific for BKV.

3.2 Biomarkers of infection

The widespread presence of BKV, JCV and MCV in a healthy population is one of the main difficulties when studying the potential association of these viruses with human cancer. Infection by these viruses is asymptomatic and mainly latent in the general population, although shedding of viral particles in urine has been observed. Reactivation of latent infection (e.g. by immunosuppression) of polyomaviruses can result in high viraemia and viruria. It is however unclear whether viruria is an appropriate biomarker for use in retrospective case–control studies, particularly when cancer (or other conditions) for which the cases are being treated, or hospitalization itself, could be associated with viral reactivation and shedding.

3.3 Notion of subtypes, variants and mutations

Genetic variants of BKV, JCV and MCV have been isolated. However, most epidemiological studies that were available to the Working Group did not consider this additional level of complexity, which may greatly influence the detection of polyomaviruses. Examples of genetic variants of each virus are described below.

3.3.1 BKV

Four BKV genotypes (I–IV) have been isolated and shown to be fully distinct serotypes (Knowles *et al.*, 1989; Pastrana *et al.*, 2012); some of the subgenotypes identified also behave as fully distinct serotypes (Pastrana *et al.*, 2013). The different BKV genotypes have different biological characteristics, and may have different cellular tropism and different pathogenic potential *in vivo* (Pastrana *et al.*, 2013).

In > 80% of BKV-positive healthy subjects, BKV belongs to genotype I, although geographical variation has been reported.

Many of the primers first developed for the detection of BKV in PCR assays were based on the sequence of genotype I and did not detect all genotypes. Consequently, variant BKV genotypes lower the sensitivity of detection by PCR and may also have a substantial effect on viral load quantitation (Randhawa *et al.*, 2011).

Most serological assays are not designed to distinguish between the four main serotypes of BKV, e.g. serum antibodies specific for BKV-I are poorly neutralizing against BKV-IV and vice versa (Pastrana *et al.*, 2012).

3.3.2 JCV

The JCV archetype and several variants classified according to rearrangements in the structure of the viral non-coding regulatory region (NCCR) have been isolated. Variants have exclusively been found in lesions associated with progressive multifocal leukoencephalopathy (PML), a JCV-induced lethal disease in immunosuppressed patients. This suggests that these JCV variants only may cause progressive multifocal leukoencephalopathy. It has been reported that in patients with progressive multifocal leukoencephalopathy, the archetype NCCR was detected in the urine, while NCCR variants were found in cerebrospinal fluid and in blood (Ryschkewitsch *et al.*, 2013). These data suggest that coincident infections with different JCV variants having different biological behaviour may occur, and highlight the importance of the choice of marker of infection.

The role of JCV NCCR variants in human cancer has received limited attention.

3.3.3 MCV

Most MCV genomes analysed so far isolated from Merkel cell carcinoma have contained mutations within large T antigen sequences leading to the expression of a *C*-terminal truncated protein deficient for viral replication. Additionally, mutations in other parts of the viral genome have been reported in Merkel cell carcinoma that may also lead to defective viral replication or assembly (e.g. within the *VP1* gene or within the origin of replication). This is in contrast to the wildtype MCV

genome commonly detected in the skin of healthy adults that expresses full-length large T-antigen and can actively produce virions.

3.4 Lytic infection versus *transformation; species specificity; permissivity and non-permissivity*

Since 1960, when SV40 was isolated and shown to induce tumours when injected into newborn hamsters, a huge amount of research has been carried out on this, the first oncovirus to be discovered, and provided many of the important paradigms in our understanding of normal cell biology and tumorigenesis (e.g. discovery of tumour suppressor genes such as *TP53* and *RB*).

The mechanism by which infection with SV40 causes cell transformation and the formation of malignant tumours is well established in rodents and in systems *in vitro*. SV40, like most polyomaviruses, gives rise to either lytic (productive) infections or incomplete (abortive) infections, depending on the host cell. Not all cells support the natural replicative and productive viral cycle and hosts can be divided into 'permissive' and 'non-', or 'semi-permissive'.

Cell transformation by a polyomavirus appears to result from an infection that fails to be lytic (productive), either because of the properties of the host cell, or because of the defective nature of the infecting virus. SV40 can easily transform mouse, rat, and hamster cells that are non-permissive for the virus, and induces tumours in these rodents. In contrast, many simian cell lines are permissive for SV40 and die when infected, liberating virions, and so cannot be transformed.

On the other hand, transformation of permissive cells is not impossible as it could result from failure of the lytic cycle owing to defects in the replicative capacity of the infecting polyomavirus. This raises the potential importance of variants of polyomavirus in human cancers (e.g. mutations of MCV large T-antigen found in Merkel cell carcinoma).

When considering the data from cancer bioassays in animals and studies in humans in its evaluation of the carcinogenicity of polyomaviruses in humans, the Working Group took into account the notions of species specificity, permissivity *versus* non-permissivity and lytic infection *versus* transformation.

3.5 Newly discovered human polyomaviruses

Since the discovery of MCV in 2008, seven additional human polyomaviruses have been identified: KI polyomavirus, WU polyomavirus, trichodysplasia spinulosa-associated polyomavirus, and human polyomavirus types 6, 7, 9 and 10. None have yet been clearly associated with human diseases, with the exception of trichodysplasia spinulosa-associated polyomavirus, which was isolated from a rare hyperplastic but non-neoplastic skin tumour that can occur in transplant patients (see "Introduction to polyomaviruses," in this Volume). Serological studies have revealed that types 6, 7 and 9 are circulating in the human population. Like the other polyomaviruses, the novel human polyomaviruses encode small and large T-antigens and thus are potentially oncogenic (Dalianis & Hirsch, 2013; Ehlers & Wieland, 2013).

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