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Polyomaviruses and Human Diseases

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DEDICATION

To the memories of Late Dr. Humayun Kabir who inspired me to study medicine and of Late Dr. Gerald Stoner, whose pioneering works inspired many scientists to study Polyomavirus.

To my parents for their everlasting support. To my family: Arzumand (wife), Shaon (daughter), and Naveed (son) for their love and sacrifice.

FOREWORD

Science never solves a pr oblem without creating ten more
Geor ge Bernard Shaw

How prophetic the above words prove to be when applied to the advances of 20th century medicine. Prior to Banting and Best, clinicians were unaware of the ravages of diabetes, patients simply wasted away and died. Following the purification of insulin, clinicians now had to deal with diabetic retinopathy, diabetic nephropathy and all the other complications of long-term diabetes. A little over 50 years ago, the first successful human kidney transplant was performed in Boston. The first 30 years of the experience had successes when compared to the alternative but were a constant struggle to get even 50% of the grafts from deceased donors to survive more than a year. However, the science continued to advance knowledge of the immune response. With this came more and increasingly powerful tools for the clinician. Suddenly, success rates of 80-90% at one year were attainable. With this success came new problems, new complications and clinicians now had to worry about the long-term consequences of their therapy as patients were surviving with functional grafts for extended periods. A particular infectious complication evolved with the application of ever more powerful immunosuppressant drugs.

Astute clinicians noted that occasionally cellular rejections seemed to get worse with steroids. Despite their best efforts and the use of powerful drugs, patients lost their grafts to overwhelming interstitial infiltrates not seen before. In the mid-1990s, investigators reported series of patients with BK nephropathy due to emergence of polyomavirus reactivation in the kidney. Polyomaviruses had been previously described and had been known to cause disease in immunocompromised hosts. These had included CNS disease due to the JC virus, association of cancer with members of the Polyoma family particularly JC and SV40, and hemorrhagic cystitis. However, the newly recognized entity of BK nephropathy began to reach epidemic proportions with some centers reporting infections in the 15-20% range. This was clearly a complication of powerful immunosuppressive drugs and sparked a renewed interest in the study of all members of this virus family.

This book is a compilation of that research explosion. The contributors have done a great service by compiling in one place the most complete collection of knowledge regarding the polyomaviruses. The clinical aspects of the disease affecting kidney transplant recipients including detection, diagnosis, monitoring and

treatment are laid out for the reader in a rational and readable manner. Polyoma virus interaction with the central nervous system as well as their association with malignancy is covered as well. A compilation of the basic science aspects of this family of viruses is gathered in one place for the first time. This work should serve those interested in these viruses and the diseases caused by them. Understanding all aspects of a disease is the first step in conquering it. Many in the field of transplantation and virology should find this book useful in future endeavors and as with any good text, it should serve as a guide and as an inspiration to those who follow.

Thomas A. Gonwa, M.D., FACP

PREFACE

"Life is short; art is long; opportunity fugitive; experience delusive; judgment difficult. It is the duty of the physician not only to do that which immediately belongs to him, but likewise to secure the cooperation of the sick, of those who are in attendance, and of all the external agents."

Oenopieles Hippocrates

What prompted this book was a seeming imbalance, between advancements made in science and what appears to be known generally. We admit that only modest progress has been made in understanding many infectious diseases such as those caused by polyomavirus. In studying infectious diseases around the world, one ends by regarding them as biological individuals, which have survived centuries, spanning human generations. Polyomavirus lends itself to such treatment because of its life cycles in the animal (human) worlds, the salient facts of which have all been elucidated within the last 30 years. However, the means of diagnosis and treatment of this virus have become, if not perfect, at least somewhat effective, and they deserve as full an explication as possible.

Many valuable monographs we possess, and even volumes of admirable papers have been published on this virus, but the former are so scattered as to be out of reach for a great number of interested readers, and the latter so academically detailed as to be unsuitable except for basic scientists. Why, then, not write a succinct manual of practice, limited to the main theme of clinical care? Ah, but an explosion of information in this field mandates both a far-reaching scope of coverage and in-depth analysis to present the complete and current picture. To meet these objectives, this book has been arranged to contain an ample outline of the history, pathology, symptoms, and treatment of diseases induced by human polyomavirus, without any detail of controversies or conflicting opinions. The use of multiple authors was essential to ensure the all-inclusive nature of this text. Many of these authors are pioneers in the field, and all have extensive experience studying or treating this virus. Because it is comprehensive, this book has broad applications for a variety of readers, including medical students, virologists, pathologists, and transplant specialists, as well as patients.

The book is divided into four sections covering the entirety of polyomaviruses from basic science considerations to clinical implications. The first section begins with a general discussion of the virus including immunology, epidemiology, and

molecular and experimental virology. Due to increasing interests in the diseases caused by Polyoma-BK virus in transplant recipients, an entire second section explores transplant-related pathobiology, histopathology, diagnosis, management, and pharmacotherapy. The third section discusses the afflictions and diseases caused by Polyoma-JC virus, particularly that of the central nervous system. The fourth section deals with neoplastic associations of polyomaviruses.

From the sketch just given, it is evident that the book has no higher goal than that of a compilation, with the addition of whatever information the distinguished authors may have from some of their own work. A few who read our book may be attracted to study the diseases that are caused by the polyomaviruses. Altogether it is earnestly hoped that the information contained in this book may be found useful, facilitating future research in this field.

We wish, as well, to recognize and honor the careers of several friends and colleagues who contributed unstintingly to research in the field of polyomavirus. To that end, the authors and editors have done what they can do, and tried to present the views and experiments of everyone, as best as they could. If any mistakes have occurred, and in a work like the present it is very possible, I shall thankfully receive notification of such errors, and shall take the earliest opportunity to correct them. If any apology be necessary for the publication of the following work, the editor accepts responsibility. Our book will be an agent of change and betterment. Even the best books have only a small effect on what people do. If our work benefits patients and draws investigators into our field, we are satisfied. That will be enough.

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CONTENTS

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High Tribute is due to those members of the Landes Bioscience staff responsible for publication. Mr. Ronald G. Landes provided strong support and advice in the preparation of all aspects of this work, and his kind invitation, encouragement and enthusiasm have been very functional in making it a reality. The contribution of Ms. Cynthia Conomos, who has been involved in all aspects of this work with an impressive commitment to detail, is gratefully acknowledged. Appreciation is also expressed to Ms. Celeste Carlton and the rest of the dedicated staff of Landes Bioscience, who skillfully processed the illustrations and prepared the thorough index.

Special recognition must be given to Professor Keerti V. Shah, who contributed the leading chapter and very importantly, gave his unconditional guidance in every aspects including selection of the contributors and the chapters. To late Dr. Gerald Stoner, Professor Giuseppe Barbanti-Brodano and my many international colleagues, my gratitude cannot be overstated.

Finally, a thoroughly dedicated colleague and my loving wife Arzumand Ara has brought her many talents in editorial preparation to the compilation of this book. Her critical and assiduous review of every chapter has been extraordinary, and her commitment and excitement in developing this book has been prime stimulus deserving of the highest praise.

Nasimul Ashsan, M.D., FACP

CHAPTER 1

Polyomaviruses and Human Diseases

Nasimul Ahsan and Keerti V. Shah

Abstract

Polyomaviruses are small, nonenveloped DNA viruses, which are widespread in nature. In immunocompetent hosts, the viruses remain latent after primary infection. With few exceptions, illnesses associated with these viruses occur in times of immune compromise, especially in conditions that bring about T cell deficiency. The human polyomaviruses BKV and JCV are known to cause, respectively, hemorrhagic cystitis in recipients of bone marrow transplantation and progressive multifocal leukoencephalopathy in immunocompromised patients, for example, by HIV infection. Recently, transplant nephropathy due to BKV infection has been increasingly recognized as the cause for renal allograft failure. Quantitation of polyomavirus DNA in the blood, cerebrospinal fluid, and urine, identification of virus laden "decoy cells" in urine, and histopathologic demonstration of viral inclusions in the brain parenchyma and renal tubules are the applicable diagnostic methods. Genomic sequences of polyomaviruses have been reported to be associated with various neoplastic disorders and autoimmune conditions. While various antiviral agents have been tried to treat polyomavirus-related illnesses, current management aims at the modification and/or improvement in the hosts' immune status. In this chapter, we provide an overview of polyomaviruses and briefly introduce its association with human diseases, which will be covered extensively in other chapters by experts in the field.

History

The polyomaviruses and papillomaviruses were previously considered subfamilies of the family *Papovaviridae*, which derived its name from three of its members: rabbit papilloma virus, mouse polyomavirus, and simian vacuolating agent or simian virus 40 (SV 40). Recently, the International Committee on Taxonomy of Viruses has recognized polyomaviruses and papillomaviruses as independent virus families. Immunologically and genetically, viruses of these two families are unrelated and also have different biological characteristics. Thirteen members of the family *Polyomaviridae* have been identified, which includes two human pathogens, JC virus (JCV) and BK virus (BKV), both of which were first isolated in 1971 from immunocompromised patients. Padgett et al[1] isolated and partially characterized JCV from the brain of a patient (with the initials J.C.) with Hodgkin's lymphoma who died of progressive multifocal leukoencephalopathy (PML), a demyelinating disorder of the central nervous system (CNS). Prior to this discovery, a virus was suspected in the etiology of PML as early as 1958; in 1969 electron microscopy of PML tissue showed viral particles in the nucleus of the infected oligodendrocytes, which were structurally identical to polyoma virion. Gardner et al[2] isolated BKV from the urine of a Sudanese renal transplant patient (with the initials B.K.) who developed ureteral stenosis and was shedding inclusion-bearing epithelial cells in his urine. Initial electron microscopy demonstrated viral particles in the urine. Inoculation of the urine into rhesus monkey kidney cells and human embryonic kidney cells produced viral cytopathic

Polyomaviruses and Human Diseases, edited by Nasimul Ahsan.

Table 1. Polyomaviruses and their natural hosts [a]

Host	Virus	Characteristics
Human	BK virus (BKV)	Early childhood infection; persists in renal epithelium and lymphocytes; causes nephropathy and ureteritis in immunocompromised hosts
	JC virus (JCV)	Late childhood infection; persists in renal epithelium, lymphocytes, and brain; causes PML in immunocompromised hosts
Monkey	Simian virus 40(SV40)	Infects Asian macaques; persists in kidney; causes PML-like disease in immunocompromised animals
	Simian agent 12 (SA-12)	Infects African baboons
	Lymphotropic papovavirus (LPV)	Multiplies in B lymphoblasts of African green monkeys
	Cynomolgus polyoma virus (CPV)	Infects Cynomolgus monkeys; persists in renal epithelium and lymphocytes; causes nephropathy and ureteritis in immunocompromised hosts, similar to BKV nephropathy in humans
Cattle[b]	Bovine polyoma Virus (BpyV)	Infects cattle; persists in kidney
Rabbit	Rabbit kidney vacuolating Virus (RKV)	Infects cottontail rabbits
Hamster	Hamster papovavirus (HaPV)	Produces cutaneous tumors in hamsters
Mouse	Mouse polyoma virus	Natural infection of wild mice and may infect laboratory mouse colonies; persists in kidneys
	K virus	Infects pulmonary epithelium of mice
Athymic rat	Rat polyomavirus	Affects parotid gland
Parakeet	Budgerigar fledging disease virus (BFDV)	Produces acute fatal illness in fledgling budgerigars

[a] Modified from reference 5. [b] A virus initially described as originating from stump-tailed macaques was subsequently identified as bovine polyomavirus

effects. The initial BKV isolate is known as the Gardner strain. In 1960, Sweet and Hilleman identified simian virus 40 (SV 40) which has rhesus macaques as the natural host.[3] Due to its ability to grow and induce characteristic cytopathic effect of cell vacuolization in African monkey kidney cells, SV 40 was initially designated as "vacuolating agent". In the late 1950s and early 1960s, millions of people were inadvertently exposed to SV 40 due to administration of SV 40-contaminated Salk polio vaccines, but this appeared to have insignificant clinical consequences.[4] Shortly, after its discovery, SV 40 was found to induce tumors in animals and to transform a variety of cell types from different species and has been periodically described to be associated with several human tumors.

Polyomavirus

Distributed widely in nature, polyomaviruses have been isolated from many species including humans (Table 1).[5] They are exquisitely adapted to grow in the species they infect and have

Table 2. Polyomavirus proteins [a]

Protein/ Region	Molecular Weight	No of Amino Acids JCV/BKV	Sequence Homology Shared with JCV[b] BKV	SV40	Function
Early coding					
Large T	79,305	688/695	83	72	Initiates viral repli cation; stimulates host DNA synthesis; modulates early and late transcription; establishes and maintains host transformation
Small T	20,236	172/172	78	67	Facilitates viral DNA replication
Late coding					
VP1	39,606	354/362	78	75	Major capsid protein; forms viral ichosahedron, enables entry,mediates hemagglutination
VP2	37,366	344/351	79	72	Minor capsid protein
VP3	25,743	225/232	75	66	Minor capsid protein; subset of VP2
Agnoprotein	8,081	71/66	59	46	Facilitates capsid assembly

[a] Modified from reference 5. [b] Percent amino acids. Molecular weight and number of amino acids of JCV proteins deduced from nucleotide data. In addition to large T and small T, a middle T antigen is coded for by mouse polyomavirus and hamster papovavirus.

probably coevolved with their hosts. Each polyomavirus infects only one or a group of closely related species.

Viral Structure and Genome

Polyomaviruses have the following properties: small size of the virion (diameter 40-45 nm), naked icosahedral capsid, superhelical double-strand circular DNA genome of molecular weight 3.2×10^6, shared nucleotide sequences with other polyomaviruses, and nuclear site of multiplication. The nonenveloped virion has icosahedral symmetry and 72 pentameric capsomers. The virion is made up of protein (88%) and a single copy of a circular double-stranded DNA molecule (12%), which has about 5,300 base pairs. BKV, JCV, and SV40 display a high degree of nucleotide sequence homology. Overall, the JCV genome shares 75% of the sequences with the BKV genome and 69% of the sequences with the SV40 genome.[5,6]

The virus-coded proteins of polyomaviruses are listed in Table 2. BKV and JCV have both species-specific and cross-reactive antigenic determinants. The viral genome is functionally divided into (i) a noncoding control region (NCCR) (0.4 kb), (ii) an early coding region (2.4 kb), which codes for tumors antigens: large T (T-ag), middle T (in mouse and hamster viruses), and small T (t-ag), and (iii) a late coding region (2.3 kb), which codes for viral capsid proteins VP1, VP2, and VP3 and agnoprotein. The NCCR is located between the early and late regions and contains the T-ag binding sites. It contains (i) the origin of DNA replication (*ori*) and (ii) the regulatory regions for early and late transcription. The sequence blocks in NCCR are arbitrarily referred to by the alphabetical designations P, Q, R, and S. These blocks serve as regulatory regions, or enhancer elements, and contain several transcription factor binding sites, which putatively modulate viral transcription.[7-12] Naturally occurring SV40, BKV and JCV strains in

the kidney and urine usually have an archetypal regulatory region. By contrast, JCV found in the brain tissue of PML usually shows a variety of point mutations, deletions, and duplications on the late side of *ori*.[13-15]

The early and late coding regions are transcribed from different strands of the DNA molecule and the direction of early and late transcription is divergent, with opposite strands participating in these processes, starting from the origin of replication.[16] T-ag is a multifunctional protein with helicase activity and distinct ability to bind host cell regulatory proteins. T-ag controls both viral DNA replication, and early and late gene transcription, and interferes with host cell transcription factors.[17] During replication, viral DNA associates with host cell histones H2A, H2B, H3, and H4 to form mini viral chromosomes, which are structurally indistinguishable from host cell chromatin.[18-21] Each pentamer of the viral icosahedron consists of five VP1 molecules and one molecule of VP2 or VP3. VP1 (molecular mass 39,600) is the major capsid protein and accounts for more than 70% of the virion protein mass. It mediates viral attachment to the receptors on susceptible cells and contains epitopes for neutralization, hemagglutination inhibition, and other virus-specific and shared immunologic determinants. VP2 (37,300) and VP3 (25,700) are minor capsid proteins.[22,23] JCV agnoprotein consists of 71 amino acid residues, with molecular weight of approximately 8 kDa. Agnoprotein differs from all other early and late proteins in that it localizes primarily in the cytoplasmic and perinuclear regions of the infected cell. Unlike viral capsid proteins, it is not detectable in the virion and its intracellular distribution has led to the suggestion that agnoprotein may promote release of virion from the cell. Agnoprotein also plays a role in the stability of microtubules and preservation of the infected cell via interaction with tubulin. BKV and JCV share a large umber of amino acids, ranging from 59% (agnoprotein) to 83% (T-ag). A greater homology exists between JCV and BKV than between JCV and SV 40.[24,25]

Isolation and Propagation

BKV can be propagated in human epithelial cells and fibroblasts. For isolation of BKV, human embryonic kidney (HEK) cells, diploid lung fibroblasts, and urothelial cells are suitable.[26] During the course of infection cytopathic effects typical of polyomavirus infections (rounding of cells containing cytoplasmic vacuoles) and formation of BKV plaques on HEK monolayers may take several weeks, whereas BKV-T antigen may be detected in infected cultures in 1 or 2 days.[27] In the case of JCV, primary human fetal glial (PHFG) cells are the most sensitive tissue culture system for isolation and propagation.[28] Human fetal Schwann cells[29] and astrocytes[30] also support JCV multiplication. Other cell types, which allow isolation of JCV, are urothelial cells, human amnion, adult brain, and HEK cells. Both BKV and JCV have been shown to produce plaques in HEK cells and can also be assayed by scoring for cytopathic effect in end-point titrations in tissue culture tubes. Because both BKV and JCV agglutinate human red blood cells of O blood type, hemagglutination can be used as a laboratory assay for quantifying virus. Both polyclonal and monoclonal antibodies to the viral T or capsid proteins are used in immunocytochemistry assays to follow the stages of BKV and JCV infection.[31-34]

Life Cycle

Depending on the host cell, polyomaviruses cause either permissive (host of origin, when all viral genes are expressed) or nonpermissive (host unrelated to species of origin) infections. All polyomaviruses multiply in the nucleus and during permissive infection the viruses cause characteristic, often pathognomonic, nuclear changes and result in cell death. Urothelial cells infected with BKV or JCV, oligodendrocytes infected with JCV, and mouse pulmonary endothelial cells infected with the mouse K virus display similar nuclear abnormalities and may result, in renal tubulo-interstitial changes, ureteral obstruction and tubular injury, PML, and pneumonia, respectively. BKV and JCV also undergo nonpermissive infection when only the viral T-ag and t-ag are made, resulting in cell transformation in tissue culture of rodent cells. In case of BKV, transformed cells exhibit BKV-T antigen and contain multiple copies of BKV-DNA. BKV-DNA is integrated into the host cell genome in rodent cells, but in human

cells, it may remain as free unintegrated copies.[35-37] The transformed cells can induce tumors in the appropriate animal hosts. Both BKV and JCV can also induce clastogenic events in infected cells, resulting in chromosomal damage, translocations, and unstable multicentric chromosomes leading to further DNA damage and ultimately cell transformation and cell death.[38]

Pathogenesis and Pathology

BKV and JCV do not naturally infect any species other than humans. The host range and tissue specificity of polyomaviruses are determined by an interaction of cellular and viral factors. There are also significant differences between BKV and JCV with respect to their biological behavior and disease potential. When inoculated into a wide variety of laboratory animals, BKV and JCV produce serologic response and sometimes tumors, but do not result in infections similar to that seen in humans. Although BKV and JCV are latent in the kidney and are reactivated in immunosuppressed states, only JCV infects the CNS and produces PML. In renal transplant recipients and in pregnant women, both BKV and JCV are reactivated frequently and are excreted in the urine; however, in bone marrow transplant recipients, BKV reactivation is far more frequent than JCV reactivation.[39,40]

The pathogenesis of a polyomavirus infection involves the following sequence of events: (1) entry of virus into the body, (2) multiplication at the entry site, (3) viremia with transport of virus to the target organs, and (4) multiplication in the target organs. VP1 interacts with specific receptors present on susceptible cells, mediates virion entry into the cell by endocytosis; virus is then transported to the nucleus, where it is uncoated.[41] BKV enters into the host cell via α (2-3)-linked sialic acids receptor. In case of JCV, an N-linked glycoprotein containing α (2-6) linked sialic acids receptor has been described on the surface of B cells and glial cells. JCV appears to enter cells by clathrin-dependent endocytosis.[42] A caveolae-dependent endocytosis allows SV40 viral entry, which requires SV40 specific receptor comprising of MHC class I and O-linked proteins.[43,44] After multiplication in the nucleus, virus reaches the target organs by the hematogenous route. The viral determinants that affect host range and tissue specificity of BKV and JCV are located in the enhancer/promoter elements in the regulatory regions[45] and the early regions of these viruses.[46,47] With respect to BKV and JCV, the route of infection is not known. Recently, JCV DNA has been isolated from tonsillar stroma and in B-lymphocyte population within the tonsils.[48] Using PCR technique, JCV DNA is routinely identified in the peripheral blood of 5-40% of normal volunteers and in brains of nearly all patients with PML.[49] While BKV is seldom recovered from the respiratory tract, the rapid acquisition of antibodies in the first few years of life is consistent with virus transmission by the respiratory route.[50] Although BKV-IgM in cord blood and BKV-DNA in fetal and placental tissues have been reported, there is controversy about the role of transplacental transmission of BKV.[51-53] Other potential sources of BKV infection are blood products, and renal allografts.[54,55] Both JCV and BKV have also been identified in other organs including heart, spleen, lung, colon, and liver. Primary infection may be accompanied by transient viruria and in the immunocompetent host, BKV and JCV persist indefinitely as latent infections.[56] BKV and JCV also persist in the kidney and B-lymphocytes for an indefinite period of time.[57,58] Reactivation of BKV and JCV in the urinary tract occurs under a wide variety of conditions, including (i) kidney and bone marrow transplantation, (ii) primary immunodeficiency diseases, (iii) immunotherapy for malignancy and other disorders, (iv) pregnancy, (v) chronic diseases e.g., diabetes, (vi) infection with human immunodeficiency virus, and (vii) old age.

SV40-associated PML in a macaque colony and SV40-associated interstitial pneumonia and renal tubular necrosis in a rhesus macaque have been reported.[59,60] In the animal with renal disease, abundant numbers of SV40 particles and large intranuclear inclusions were seen in the renal tubular epithelial cells. The disease was similar to BKV-induced tubulointerstitial nephritis, described in a child with an inherited immunodeficiency disease.[61] SV40-associated PML occurred in immunosuppressed simian immunodeficiency virus (SIV)-infected rhesus macaques.

In a kidney transplant model, Gorder et al[62] described a new polyomavirus (cynomolgus polyoma virus—CPV) from renal tubules of cynomolgus monkeys (*Macaca fascicularis*) treated with cyclosporine and azathioprine. This virus has 84% DNA sequence homology to SV40. Most of the animals infected with polyomavirus developed lethargy, anorexia and had rising serum creatinine due to polyomavirus interstitial nephritis in the native kidney and/or the renal graft. In addition, several grafts had extensive rupture and destruction of collecting ducts and demonstrated endarteritis and focal hemorrhage indicative of active cellular rejection. None of the animals with detectable virus in the allograft had infections of the native kidney. In the renal graft, the peak frequency of infection was from day 21-48 after transplant and during this study, no particular association of polyomavirus with any of the immunosuppressive agents was evident.

Clinical Features

Primary Infection

In healthy children, primary infection with BKV and JCV is rarely associated with clinical disease. In a prospective study, 11 out of 66 children with respiratory illness demonstrated BKV seroconversion; seven of these children had mild respiratory disease and four were asymptomatic. BKV was isolated from the urine of one of the children showing seroconversion. Unintegrated BKV DNA was identified in the tonsillar tissue of five of 12 children with recurrent respiratory disease.[63] In immunocompromised children, primary BKV infection may cause cystitis or nephritis and primary JCV infection may lead to PML. Primary BKV infection may also present with encephalitis. Following primary infection viruses persist indefinitely as "latent" infections of the kidney.

Silent Viruria

BKV can be reactivated after many years, usually by states of acquired (cell-mediated) immunosuppression: pregnancy, HIV, neoplasm, systemic lupus erythematosus, nephrotic syndrome, bone marrow, and organ transplantation. Twenty per cent of immunocompetent patients are found to have JC viruria; in this situation whether viral shedding represents reactivation or new infection remains unclear.[64]

Pregnancy

Approximately 3.2% of pregnant women during second (late) and third trimesters show cytologic evidence of BKV and JCV excretion in urine.[65] In tests of paired sera spanning pregnancy, a rise in antibody titers to BKV or JCV was found in 14% of the women.[66] The viral reactivation may be induced by hormonal changes and shedding continues intermittently through the pregnancy until the postpartum period. While controversy exists about trans-placental transmission to fetus, viral excretion does not appear to be associated with any ill effect to the mother.[67]

Systemic Lupus Erythematosus (SLE)

The prevalence of BKV genome is significantly higher than JCV in the serum of patients with SLE.[68] Christie et al[69] observed that rabbits inoculated with BKV particles produced antibodies directed to both viral structural protein and host histones. It has been suggested that BKV infection may contribute to the development of SLE as supported by the findings that patients with BKV infection with expression of large T-ag develop anti-DNA antibodies and anti-BKV antibodies have some cross reactivity with DNA.[70] Indeed, using PCR, Sundsfjord et al[71] have identified BKV genomic sequences in 16% of 44 patients with SLE. In another study, 80% (16/20) of SLE patients showed at least one or several episodes of BKV (12 patients) or JCV (4 patients) reactivation, while control group did not have any viral replication.[72] Similarly, several other investigators reported that reactivation of polyomavirus in

patients with auto-immune diseases including SLE.[73,74] In these reports, observed viruria was found to be independent of immunosuppressive therapy suggesting that an unknown inherent immunologic defect in SLE patients might be the contributing factor. In this book, Rekvig and colleagues have discussed the association between polyomavirus and auto-immunity in great detail in a subsequent chapter.

HIV Disease and Polyomavirus

The major polyomavirus associated disease in HIV infected patients is JCV mediated PML (*vide infra*) which occurs in about 1.6% of the cases. The role of polyomavirus infection in kidneys was examined in a retrospective study using immunohistochemistry for T-ag and PCR.[75] Multifocal polyomavirus replication was diagnosed in 6.3% (7/111) of the patients. Cytopathic changes of limited necrosis, interstitial infiltrates, and intratubular casts were noted. Surprisingly, JCV genomes were identified in five of these seven patients. Several studies have demonstrated that 20-30% of patients with HIV disease also excrete BKV in the urine without any symptom.[76-78] The frequency of BK viruria increases with decreasing CD4 count when the prevalence of BKV shedding increases from 4-8% to 27-51% when CD4 counts fall below 200/μL.[76,78,79] Despite frequent reactivation in AIDS, clinical manifestations BKV are rare. In patients with AIDS, BKV has been reported to cause fatal tubulointerstitial nephropathy, disseminated pulmonary infection, retinitis, and meningoencephalitis.[78,80-82] Others have reported hemorrhagic cystitis similar to that observed in bone marrow transplant recipients.[83,84]

Renal Transplant Recipients—Polyomavirus-Associated Nephropathy

Infections in renal transplant recipients have been studied by several investigators[85-91] and have been frequently reviewed.[92-103] In a multicenter serologic study of nearly 500 renal allograft recipients in the United States, BKV and JCV infections occurred, in 22% and 11% of the patients, respectively.[88] Coexistence of SV40 infection has also been described.[104] Virus shedding in urine of renal transplant recipients has been monitored by a variety of techniques, including urinary cytology, immunoassay, electron microscopy, virus isolation, ELISA assays, nucleic acid hybridization, and PCR.[105-119] In prospective studies, 25-44% of renal transplant patients excrete virus in their urine in the posttransplant period. The duration of excretion ranges widely, from transient viruria to excretion over several weeks or several months. The kidney of a seropositive donor may initiate infections in the recipient.[120] Infections may be either reactivations or primary infections affecting up to 5% of renal allograft recipients in about 40 weeks (range 6-150) post-transplantation. More than 50% of the patients show serologic evidence of infection with the virus. Persistent BKV infections have been associated with irreversible graft loss in more than 50% of the cases over 12-240 weeks of follow-up.[97,117] The infections appear to be responsible for some of the cases of ureteral obstruction.[121,122] Risk factors include treatment of rejection episodes and increasing viral replication under potent immunosuppressive drugs such as tacrolimus, sirolimus, or mycophenolate mofetil.[92,94-98,101,123] The histological presentation of BKV nephropathy has been described recently[88,94,95] (Fig. 1). Cytopathic changes in renal tubules reflecting viral multiplication consist of enlarged nuclei with smudgy chromatin, intranuclear inclusions, rounding and detachment. These have been classified into: (a) stage A: focal medullary involvement of tubular cells, (b) stage B: extensive renal involvement with multifocal or diffuse cytopathic alterations, necrosis, profound inflammatory response, and early fibrosis, and (c) stage C: characterized by interstitial fibrosis, scarring, and calcification.[98] BKV related vasculopathy, a new tropism, has been described recently, in which a fatal case of disseminated BKV infection in a renal transplant recipient was associated with BKV multiplication in endothelial cells.[124] In subsequent chapters, several authors have also discussed polyomavirus related infections particularly in the setting of organ transplantations.

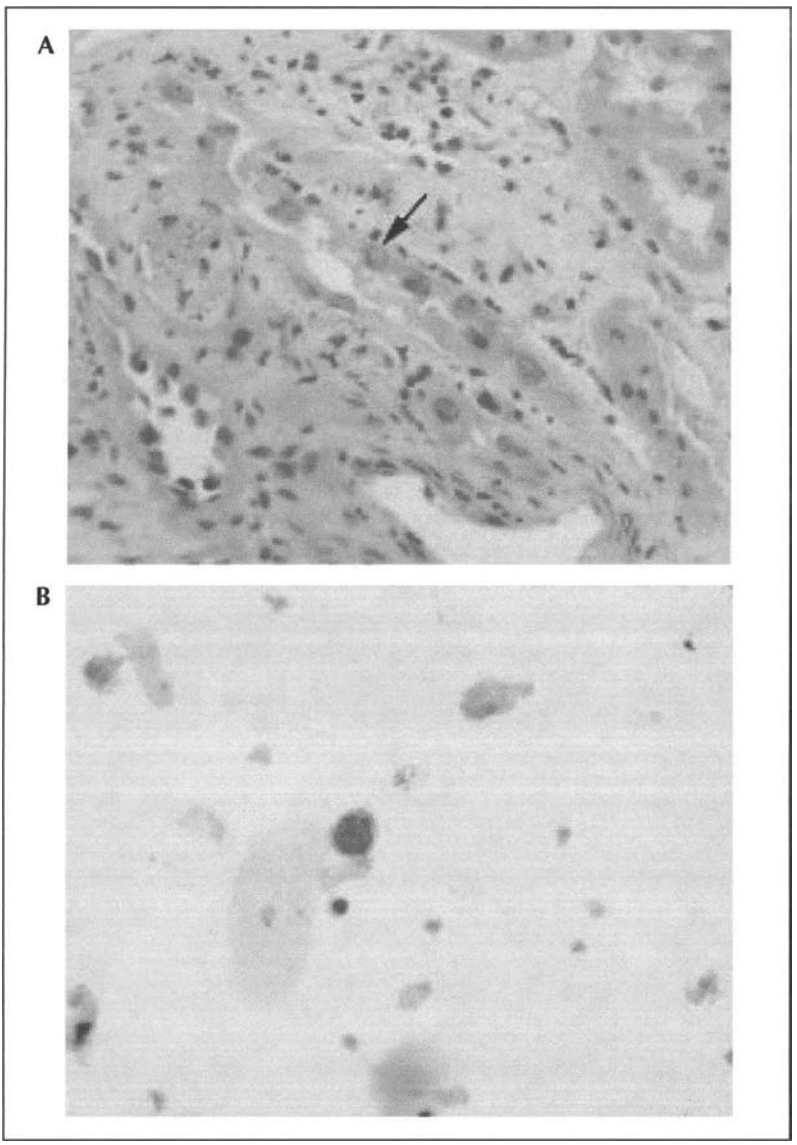

Figure 1. BKV nephropathy: A) Light microscopy of transplant kidney tissue: BKV laden renal tubular epithelial cells with intranuclear inclusion (hematoxylin-eosin stain, x 200). B) Urinary cytology showing BKV infected urothelial cell, so called "decoy cell". Note rounded nucleus with smudgy, glassy intra nuclear inclusion and margination of nuclear chromatin (Papanicolaou stain, x 400).

Bone Marrow Transplantation and Hemorrhagic Cystitis

Hemorrhagic cystitis (HC) is not an uncommon complication affecting more than 10% of the recipients of bone marrow transplantation (BMT).[125-128] Transient HC occurring in the first few days after transplantation usually represents drug toxicity. About one-half of the BMT patients shed BKV without any symptoms of HC in the posttransplant period, which is higher in recipients of allogeneic marrow. Late onset HC (2-12 weeks post-transplant) that lasts more