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Polyomavirus BK and Autoimmunity to Nucleosomes

Ole Petter Rekvig and Ugo Moens

Autoimmunity to DNA and nucleosomes has been regarded as central to understanding the nature of the autoimmune syndrome systemic lupus erythematosus and organ manifestations typical of this disorder. Particularly, glomerulonephritis is a serious manifestation where antibodies to dsDNA are believed to be a causal factor. The genesis of autoantibodies to dsDNA has been an enigma for more than four decades, and critical insight into processes operational in vivo that account for this antibody production are still lacking. It was discovered some years ago that polyomaviruses had the potential, when injected into experimental animals, to initiate the production of anti-dsDNA antibodies and T cell immunity to nucleosomes. The authors provide evidence that these viruses initiate autoimmunity to nucleosomes by a process where viral DNA binding proteins bind host cell nucleosomes, rendering this complex immunogenic, not only in autoimmune-prone animals, but also in healthy ones. This review describes the processes involved in this particular virus-induced autoimmunity.

ABBREVIATIONS:

APC	Antigen presenting cells
LT-ag	Polyomavirus large T-ag
ssDNA	Single-stranded DNA
dsDNA	Double-stranded DNA
VP1-3	Viral capsid proteins 1-3

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Introduction

BK virus (BKV) was first isolated from the urine of a Sudanese patient with the initials B. K. who had received a renal transplant from his brother. Electron microscopy examinations of urine samples of this patient and of cell cultures inoculated with urine specimens revealed the presence of polyomavirus-like particles. The virus agglutinated erythrocytes, which distinguished it from the closely related monkey polyomavirus SV40 that was known at that time, and the virus was therefore classified as a new polyomavirus. BK virus is now classified as a human polyomavirus. The approximately 5100 base-pairs dsDNA genome has a tripartite functional organization. The noncoding control region, containing the origin of replication and the promoter-enhancer sequences, separates the early and late regions. The early region encodes the large T antigen (LT-ag) and small t antigen (ST-ag) proteins, whereas the agnoprotein and the capsid proteins VP1 to VP3 are encoded by the late region. The LT-ag binds both viral DNA and cellular chromatin and plays a pivotal role in viral DNA replication and transcription. Both LT-ag and ST-ag can exert pleoitropic effects on biological processes in the host cell such as DNA replication, cell cycle progression, and gene expression.[1]

Worldwide conducted serological surveys revealed that BKV is ubiquitous and has a high incidence among humans.[2] Primary infection occurs predominantly during childhood and seems, with few exceptions, to be subclinical. After primary infection, the virus normally remains in a lifelong latent persistence in immunocompetent individuals.[3] The knowledge of the molecular biology of this virus has expanded extensively during the past years. Still, however, three decades after its original isolation, our understanding of important biological aspects of BKV infections in humans remains limited. Very little is known about the routes of infection and transmission, the host cell specificity, the mechanisms for spreading and reactivation, and the etiological role of BKV in human diseases. Detection of viral proteins or DNA sequences in organs and body fluids of healthy individuals and patients, clinical conditions that lead to viral reactivation, and diseases association with BKV may contribute to our understanding on the biolo-

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SLE:

Systemic lupus erythematosus, as classified by Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. **Arthritis Rheum 1982;25:1271-7**.

AUTOIMMUNITY:

B cells and/or T cells that respond to true autologous ligands, such as self proteins or nucleic acids.

gy of BKV. Table 1 summarizes the human tissues and fluids in which viral proteins or sequences have been detected under different clinical conditions. BKV is often reactivated in immunocompromised individuals. We have detected an unexpected consequence of primary BKV infection or after BKV reactivation. Initially, this consequence was noted after immunization of rabbits with BKV in order to prepare antibodies to the BKV structural proteins. This immunization resulted in the production of antibodies not only against the virus but also against host cell histones and dsDNA.[4] The latter antibodies are still regarded as hallmarks for autoimmune diseases such as systemic lupus erythematosus (SLE).[5] Such results add weight to the discussion of a viral etiology for SLE or SLE-related autoimmunity.[6] This initial observation therefore prompted us to investigate a role of BKV in SLE. In the next section, this autoimmune consequence of clinical polyomavirus infection will be presented, opening up a new role for polyomaviruses in clinical medicine.

Anti-DNA Antibodies: An Introduction

Hardly any single naturally produced autoantibody population has been subjected to such a wide scientific interest as antibodies to DNA, particularly to mammalian B-helical dsDNA. Forty years of intensive research have resulted in testable and verified models related to the cellular and molecular bases for the origin and production of antibodies to dsDNA. Our contribution to this improved knowledge is that polyomaviruses may be linked to one pathway for rendering DNA and nucleosomes immunogenic in vivo. However, this new and critical insight also resulted in the generation of novel and serious questions about the function and biological consequences of their de facto existence. Although we may understand the basis for the generation of these antibodies, implying the perception that all human individuals have the potential to produce antibodies to dsDNA, it is still difficult to explain why immunocompetent individuals do not produce such antibodies. The purpose of this review, however, is to describe how polyomaviruses such as BK may initiate and maintain the production of antibodies to DNA, particularly to mammalian dsDNA.

Key Points

- Polyomaviruses infect the human body and can be detected in diverse organs and tissues throughout the body.
- Primary infection is normally followed by a lifelong latent infection but reactivates in systemic lupus erythematosus.
- Large T antigen is encoded by the viral genome, and its expression is a prerequisite for virus replication.
- When expressed, large T antigen binds host cell nucleosomes, thus creating a self (nucleosomes)/nonself (large T antigen) complex.
- Nucleosome large T antigen complexes have the potential to antigen selectively stimulate DNA-specific B cells and nontolerant large T antigen-specific T cells.
- This cognate interaction model may account for the production of potentially pathogenic SLE-related antibodies to double-stranded DNA.

DNA Selects and Expands DNA-Specific B Cells

At the time we became interested in the origin of antibodies to DNA, mammalian dsDNA itself was hardly accepted as possessing immunogenic potential. We, as well as others, challenged this view and asked which requirements should be fulfilled for DNA to be rendered immunogenic. The alternatives at that time were several. Either DNA itself or a cross-reacting antigen initiated the antibody production. Furthermore, by accepting DNA as the driving antigen, two options were given to explain how DNA could act as an antigen in vivo. DNA could be a complete antigen or alternatively act as a hapten. Because experimental proof supporting the first option is lacking, DNA in our opinion should be complexed to a nonself polypetide to operate as a full immunogen. Therefore, we found it more fruitful to search for proteins (or other ligands) that render DNA immunogenic rather than searching for immunogenic DNA.[7]

During the past decade, this approach has proven extremely fruitful in explaining not only how these

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Table 1 | **A Survey of Polyomavirus BK Infectivity and Organs in Which BK Virus Is Detected**

Tissue/Fluid/Cell Type	Clinical Condition	Viral Component	Method of Detection of Polyomavirus BK	References[a]
Bladder and ureter	Patient with malignancies	Large T-ag	IHC	1, 3, 41, 42
	BMT patient	Virions	Cell cultures[b]	1, 3, 42
	Normal tissue	Early region sequences	PCR	1, 41, 42
	Renal allograft recipients	Virions, viral proteins, BKV DNA	EM, IHC, PCR	1, 3, 41
	Patient with AIDS and non-Hodgkin's lymphoma	BKV DNA	PCR	53
Blood	Measles patients	Virions	EM	3
	AIDS patients	BKV DNA; early region, NCCR, and VP1 DNA; virions	PCR, nPCR, cell culture	1-3, 41, 42, 53
	Healthy subjects	Early region, NCCR, VP1, and late region DNA; early transcripts	PCR, nPCR, RT-PCR, ISH, hybridization	1-3, 41, 42
	Renal allograft recipients	Early region and VP1 DNA	PCR, nPCR	1-3, 41, 42-44, 46, 47
	BMT	Early region, NCCR, and VP1 DNA	PCR, nPCR	1-3, 42, 47
	Patients with neural disorders	Early and late region DNA	PCR, hybridization, ISH	1-3, 41, 42
Bone	Normal bone tissue	Early region DNA and transcripts	PCR, RT-PCR	1, 3, 42
Bone marrow	AIDS patients	BKV DNA, virions	PCR, cell cultures	1, 3, 41, 42, 53
Brain	Aborted fetuses	NCCR and VP1 sequences	nPCR	1-3, 41, 42
	AIDS patients	BKV DNA, agnoprotein, capsid proteins, virions	PCR, nPCR, ISH, IHC, EM	1, 3, 41, 42, 53
	Patient with malignancies	NCCR, agno, VP1, T-ag DNA sequences, virions	PCR, EM, cell cultures	1, 41, 56
	Normal brain tissue	Early region DNA and transcripts	PCR, RT-PCR	2, 3, 41, 42
	Patients with multiple sclerosis	Early region DNA	PCR	1-3, 41, 42
	Patients with Huntington's disease	PCR	1-3, 41, 42	
	Patients with systemic nonneurological, nonmalignant diseases	Early region DNA	PCR	1-3, 41, 42
	Non-PML-affected brain tissue	Early region DNA	PCR plus hybridization	3, 42
Central nervous system	AIDS patients	BKV DNA, virions, and viral proteins	nPCR, ISH, cell cultures, IHC, hybridization	1, 3, 41, 42
Cerebrospinal fluid	Patient with encephalitis	Early region DNA	PCR	1-3, 41, 42
	AIDS patients	BKV DNA, early region and NCCR DNA, virions, viral proteins	PCR, nPCR, EM, ISH, IHC, hybridization, cell cultures	1-3, 41, 42, 53
	Patient with malignancies	NCCR, agno, VP1, and T-ag DNA sequences; virions	PCR, cell cultures	56
Cervix and vulva	Healthy individuals	Early region DNA	PCR	1, 3, 41, 42
Connective tissue	Normal tissue	BKV DNA	Hybridization	1, 41
Endothelial cells	Renal transplant patient	Early region DNA and viral proteins	PCR, IHC	52
Eye	AIDS patients with encephalitis, nephritis, and retinitis	Early region and VP1 sequences, agno and VP1 proteins	nPCR, IHC	1, 3, 41, 42
Heart	Renal transplant patient	Early region DNA	PCR, hybridization	52
Kidney	Normal kidney tissue	Early region, BKV DNA	Hybridization, PCR	1-3, 41, 42
	Patient with malignancies	NCCR, agno, VP1, and T-ag DNA sequences; BKV DNA; virions; viral proteins	PCR, cell cultures, hybridization, IHC	1, 3, 41, 42, 56
	AIDS patients	Early region DNA, agno and VP1 proteins, virions, BKV DNA	nPCR, IHC, EM, LM, hybridization, ISH	1, 3, 41, 42, 53
	Renal transplant patients	Virions, viral proteins, BKV DNA, early region, NCCR, VP-1, and VP1-T-ag DNA sequences	EM, IHC, ISH, PCR, nPCR, LM	1, 2, 42, 45-47, 57
	Renal and pancreas transplant patients	Viral proteins	IHC	45
	Pancreas transplant patient	Virions, viral proteins	LM, EM, IHC	58
	6-year-old boy with IgM hyperimmunoglobulinemia	Viral proteins	IHC	1, 42
	Aborted fetuses	NCCR and VP1 DNA	nPCR	1-3, 41, 42
Lips	Normal tissue	DNA sequences	PCR	41
Liver	Normal liver of a patient with a liposarcoma and of a patient with carcinoma of bladder	BKV DNA sequences	Hybridization	1, 3, 41, 42
Lung	Patient with malignancies	NCCR, agno, VP1, and T-ag DNA sequences	PCR, cell cultures	56
	Normal lung tissue	BKV DNA	Hybridization	41
	BMT patient	VP1 sequences	PCR	1, 3, 42
	6-year-old boy with IgM hyperimmunoglobulinemia	DNA sequences	Hybridization	1, 42
	AIDS patients	Viral proteins, virions, viral DNA sequences	EM, IHC, ISH, hybridization	1, 3, 41, 42
Lymph nodes	6-year-old boy with hyperimmunoglobulin M immunodeficiency	BKV DNA	Hybridization	1, 42
	HIV-1-positive patients with AIDS	Early region DNA	PCR	41, 42

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Table 1 (continued)

Tissue/Fluid/Cell Type	Clinical Condition	Viral Component	Method of Detection of Polyomavirus BK	References[a]
Mesentery	Patient with AIDS and non-Hodgkin's lymphoma	BKV DNA	ISH	53
	Patient with carcinoma of the pancreas	BKV DNA	Hybridization	1, 41
Penis	Healthy individuals	Early region DNA	PCR	1, 3, 41, 42
Pituitary gland	Normal tissue	Early region DNA	PCR	42
Placenta	Women with normal pregnancy	NCCR and VP1 sequences	nPCR	1-3, 41, 42
Prostate	Normal tissue	Early region DNA	PCR	41
Saliva	AIDS patient	Virions	Cell cultures	1, 3, 41, 42
Skin	Healthy individuals	Early region DNA	PCR	1, 3, 41, 42
Small intestine	Normal tissue of a patient with carcinoid tumor	BKV DNA	Hybridization	1, 41
Sperm	Healthy individuals	Early region DNA	PCR	1, 3, 41, 42
Spleen	6-year-old boy with IgM hyperimmunoglobulinemia	BKV DNA	ISH	53
	Normal tissue	BKV DNA	Hybridization	41, 42
Stomach	Patient with AIDS and non-Hodgkin's lymphoma	BKV DNA	ISH	53
Tongue	Normal tissue	DNA sequences	PCR	41
Tonsils and nasopharynx	Children with respiratory diseases	Early and late region DNA, NCCR, BKV DNA	PCR, hybridization	1-3, 41, 42
	Throat washing of a healthy individual	VP1 DNA sequences	PCR	2, 41, 42
Urine	Renal allograft recipients	Virions, viral proteins, "decoy" cells, BKV DNA, and early region, NCCR, and VP1 DNA	LM, EM, immuno-EM, dot ELISA, IHC, hybridization, PCR, nPCR, ISH, cell cultures[b]	1-3, 41-46, 47, 48
	BMT	Virions, viral proteins, "decoy" cells, BKV DNA, and early region, NCCR, agno, and VP1 DNA	LM, EM, immuno-EM, IHC, PCR, nPCR, hybridization, cell cultures	1-3, 41, 42, 47-52
	Patients with malignancies	Virions, viral proteins, "decoy" cells, and early, NCCR, agno, and VP1 DNA	EM, dot ELISA, PCR, nPCR, IHC, cell cultures, LM	1-3, 41, 42
	AIDS patients	Virions, viral proteins, "decoy" cells, and early, NCCR, agno, and VP1 DNA sequences	EM, dot ELISA, PCR, nPCR, IHC, cell cultures, LM	1-3, 41, 42
	Pregnant women	"Decoy" cells; virions, NCCR, and VP1 DNA sequences	LM, EM, PCR, nPCR, ISH, cell cultures	1-3, 41, 42
	Healthy individuals	"Decoy" cells; virions, NCCR, early region, and VP1 DNA sequences; viral proteins	dot ELISA, EM, ISH, IHC, hybridization, PCR, nPCR, cell cultures	1-3, 41, 42
	Patients with autoimmune diseases	NCCR and VP1 DNA sequences	PCR, nPCR, direct cloning, and sequencing	1-3, 41, 42
	Patients with undefined clinical conditions	"Decoy" cells, viral proteins, and NCCR, VP1, VP2, and BKV DNA sequences	PCR, nPCR, LM, hybridization, cloning, IHC	1-3, 41, 42, 54
	Child with nephrotic syndrome	Virions, viral proteins	EM, IHC	1
	Patients with hepatic diseases	"Decoy" cells, virions, viral proteins, BKV DNA	LM, EM, ISH, PCR	1, 2
	Liver transplant patients	Early region DNA, large T-ag	IHC, PCR	2
	Heart transplant patient	Virions, large T-ag, and early region, NCCR, and VP1 DNA	EM, ISH, PCR, IHC	1, 2, 42
	Patients with CNS disorders	BKV DNA, virions	EM, hybridization, IHC, cell cultures	2, 3
	6-year-old boy with IgM hyperimmunoglobulinemia	Viral proteins	IHC	1, 42
	Children with cystitis	Virions, viral proteins, early region DNA	EM, IHC, PCR	1-3, 41, 42
	Patients suffering from renal diseases	"Decoy" cells, virions, BKV DNA	LM, EM, ISH, PCR	1, 2
	Patients with inflammatory diseases	"Decoy" cells, virions, BKV DNA	LM, EM, ISH, PCR	1, 2
	Patients with urinary tract symptoms	"Decoy" cells, BKV DNA	LM, hybridization	1, 2
	26-year-old male suffering from hyperthyroidism	"Decoy" cells, virions, viral proteins	LM, EM, IHC	55
	Child with acute tonsillitis	Virions, BKV DNA	EM, restriction enzyme analysis of DNA, IHC	1-3, 41, 42
	Diabetic patients	"Decoy" cells, virions, BKV DNA	LM, EM, ISH, PCR	1, 2, 42
	18-year-old girl suffering from Fanconi's anemia	Virions	EM	41

BMT, bone marrow transplant patient; EM, electron microscopy; IHC, immunohistochemistry; ISH, in situ hybridization; LM, light microscopy; NCCR, noncoding control region; PCR, polymerase chain reaction; nPCR, nested PCR; RT-PCR, reverse transcriptase-PCR; PML, progressive multifocal leukoencephalopathy; CNS, central nervous system.
a. Primary sources are included in these references.
b. Viral rescue after inoculation onto cell cultures.

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antibodies may be initiated but also how they may be maintained. Thus, by regarding DNA as a hapten, this would work as a complete immunogen only in the context of a carrier protein serving as a potential T helper cell stimulus. This requisite would entirely explain the bases for both the immunoglobulin class switch and for somatic mutations (affinity maturation) along the progressive immune response, as had been noticed among spontaneously produced anti-dsDNA antibodies in SLE.[8,9] DNA-binding methylated bovine serum albumin (mBSA) had been used for several decades as a carrier protein for DNA. This was successful for most DNA structures but not for mammalian dsDNA.[10,11] Therefore, the dogma slowly precipitated that mammalian dsDNA was not immunogenic, not even in the context of an immunogenic carrier protein. Now, in light of later observations using other carrier proteins (see below), the results using mBSA as a carrier protein are difficult to explain but have forced the investigations on DNA immunogenicity into derailment. Thus, because DNA in controlled experiments could not induce production of antibodies to dsDNA, these antibodies were assumed to be the result of diverse processes such as polyclonal B cell activation, cross-stimulation by structurally similar antigens, or somatic mutation of structurally related antibodies, thus giving rise to specificity to dsDNA through the concept of activation by antibody idiotypes.[5] None of these pathways satisfactorily explained the sustained production of antibodies to dsDNA, although they may to a certain degree account for the initiation of these antibodies. These alternative views may provide the best explanation why this field has progressed so slowly: they most probably did not represent the biologically relevant pathways for sustained production of anti-dsDNA antibodies, are difficult to prove, and are therefore technically and intellectually demanding.

Autoimmunity to DNA: The Impact of In Vivo Produced Nonself Proteins

Indirectly, from studies of the structures of the V region of both IgM and IgG anti-dsDNA antibodies demonstrating a continuous (intraclonal) selection of somatically mutated structures favoring binding of the antibodies to dsDNA, all available data generated strongly indicate that the antibodies were driven by DNA and that they were T helper cell dependent. For several years, direct evidence for this possibility was missing. In the beginning of the 1990s, two laboratories published data that established that DNA in fact could induce and maintain the production of anti-DNA antibodies. Common for these laboratories was that they demonstrated the need for an immunogenic carrier polypeptide complexed with DNA.

Data published by Desai and colleagues[12] experimentally proved that DNA could act as an immunogen when complexed with a synthetic peptide of a protein encoded by *Trypanozoma crucii*, called Fus1. Antibodies produced in response to this complex were highly similar to those detected in SLE with regard to fine specificity for mammalian dsDNA, as well as to the structures of their heavy and light chain variable regions.[13] These data were important as they solved a long-standing problem; they reflected DNA as the antigen and proved that DNA really had the potential to induce such antibodies.

At the same time, we published an experimental system demonstrating another biological process that terminated B cell tolerance to DNA. This process was linked to in vivo expression of polyomaviruses[4,14-16] and generated a description of a natural biological process that was involved in anti-DNA antibody production. This model, experimentally easy to approach,[4] could be pursued among patients producing anti-DNA antibodies.[17] Moreover, the model could be used to describe both the molecular (generation of immunogenic DNA-protein complexes in vivo) and cellular (specificity of both B cells and T cells involved in the generation of anti-DNA antibodies) processes that were operational in a physiological and biological context.[7] In the following section, the main observations and conclusions within the frame of this experimental system are reviewed.

Polyomavirus BK and B Cell Autoimmunity to DNA

Deriving from the idea that nonself DNA-binding proteins could have the potential to render DNA immunogenic, we assumed viruses to be candidates that express such proteins. Indeed, Christie et al.[18] observed that rabbits inoculated with infec-

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tious polyomavirus BK particles produced antibodies not only to viral structural proteins but also to histones. This initial observation encouraged us to test whether such sera also contained antibodies to DNA. This was indeed the case,[14] and it generated aimed investigations on the processes that could explain why and how BK viruses were inflicted in this autoimmune response. Two options were given by this experiment to explain why such autoantibodies were produced. The virus could act as a polyclonal B cell activator or—in our opinion, a more attractive idea—the virus encoded DNA-binding proteins that could give immunogenic potential to DNA and nucleosomes.

For example, human polyomavirus LT-ag binds viral DNA as well as cellular nucleosomes.[1] This latter property could theoretically permit LT-ag to bind DNA and consequently render the DNA-histone complexes immunogenic for both B cells and T cells.[19-21] This assumption was subsequently tested by injecting immunologically normal mice with plasmids expressing the single protein SV40 or BK virus LT-ag under the control of a constitutively active promoter.[17,22] Expression of the plasmids was verified by the production of antibodies to LT-ag, and the impact of this expression on autoimmunity to DNA was demonstrated by a timely linked production of IgG antibodies to DNA.[22] Thus, DNA vaccinations of mice with plasmids encoding the SV40 or BK virus large LT-ag resulted in the production of antibodies to ssDNA, dsDNA, histones, and LT-ag.[22] On the other hand, mice vaccinated with a control plasmid containing the LT-ag coding sequences but lacking a functional promoter or a plasmid expressing the non-DNA-binding protein luciferase did not develop antibodies to components of nucleosomes, although in the latter case, antibodies to luciferase were induced.[22] Those results indicated that induction of anti-DNA and anti-DNA-binding protein antibodies required that the expressed nonself protein bind nucleosomes in vivo and furthermore proved that plasmid DNA by itself did not account for the generation of anti-DNA antibodies. This finding was in line with the proposed mechanism for how the BK virus could initiate autoimmunity to DNA. The idea that expression of a viral DNA-binding protein, when bound to nucleosomes, initiated the production of anti-DNA antibodies formed a novel physiological explanation for how such antibodies may be produced. Linked to this assumed process, we suggested that LT-ag acted as a carrier protein for DNA contained in the nucleosomal complex. As DNA is not naked in vivo, this would imply that LT-ag also could act as a carrier protein for other autologous ligands bound to the basic nucleosomal complex. In agreement with this assumption, we also observed antibodies to the DNA-binding eukaryotic transcription factors TATA-binding protein (TBP) and to the cAMP response element–binding protein (CREB) but not to irrelevant nuclear or cytoplasmic antigens in mice inoculated with the LT-ag expressing plasmid.[17] From these data, we predicted that polyomavirus expression in vivo might be linked to production of antibodies to DNA, irrespective of the clinical situation. The predictions derived from the experimental model described above have been confirmed in our laboratory in two different clinical approaches, which are outlined below.

Polyomavirus and Anti-DNA Antibodies: Clinical Observations

As primary infection with the BK virus usually takes place during early childhood, we tested a series of serum samples from children for the development of antibodies to viral structural proteins and to DNA. Seroconversion, as defined by the appearance of antibodies to the viral structural proteins, was taken as indication for primary infection. Parallel with the development of antibodies to the structural proteins, we could detect a transient production of antibodies to DNA, including weak antibodies to dsDNA, as determined by ELISA. Thus, in otherwise healthy children, transient antibodies to DNA were detected concomitantly with the primary infection.[15]

In another study, we followed 20 SLE patients for 1 year. During this year, urine samples were examined by polymerase chain reaction (PCR) for polyomavirus DNA, and serum samples were assayed for antibodies to LT-ag, DNA, TBP, and CREB. BKV viruria was observed in 12 patients, and another 4 patients secreted JCV in their urine specimens. No temporal relationship between immunosuppressive therapy and episodes of viruria could be detected. Sequencing the noncoding control region

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revealed that 11 of 12 patients secreted viruses of the archetypal BKV WW strain. This strain is circulating in the normal human population. The BKV isolates with the WW regulatory region remained stable over an observation period of 1 to 3 years (mean = 22 months). This, as well as the fact that no signs of viremia were observed in any of the SLE patients, suggested reactivation of the virus rather than a primary or secondary infection in these patients. Serum samples of these 16 patients contained antibodies to DNA and LT-ag.[17] In 2 patients, we could directly measure development of anti-DNA antibodies following virus reactivation. However, in 1 patient, anti-DNA antibodies were produced constitutively, although no signs of virus shedding or antibodies to LT-ag could be detected,[17] thus clearly demonstrating that other pathways than that related to polyomaviruses are operational for the production of antibodies to DNA.

In a recent cross-sectional study, we analyzed single serum samples from patients suffering from SLE, Sjøgren syndrome, rheumatoid arthritis, or unclassified connective tissue diseases, in addition to serum samples from healthy blood donors, for antibodies to DNA, LT-ag, and TBP. We observed a statistically highly significant correlation between antibodies to LT-ag, DNA, and transcription factors such as TBP but not to rheumatoid factors.[23] Thus, spontaneous polyomavirus reactivation with requisite LT-ag expression may provide at least one molecular basis for the initiation of autoimmunity to DNA and to other nucleosome-associated antigens.

Polyomaviruses and T Cell Autoimmunity to Nucleosomes

As described above, data have been generated that firmly establish that DNA is immunogenic for B cells and that, for example, LT-ag-specific T cells provide helper stimulus for such B cells (see Fig. 1 for details). However, in SLE, autoimmune T cells seem to be operational in cognate B cell–T cell interaction, as it is established that in this syndrome, activated autoimmune histone- and nucleosome-specific T cells may provide help for DNA-specific B cells.[24-26] From contemporary knowledge, one may ask whether an operational link may exist between activation of LT-ag-specific and histone-specific T cells.

One way this may occur may be through a process implying linked presentation of nonself and self molecules by the same antigen producing cell (APC), provided that T cells reactive to nonself molecules are present.[20] Precedence for this model relates to diversification of T cell proliferations to include responses to determinants between different polypeptides.[27-29] For example, Lin et al.[28] observed that autologous cytochrome C was nonimmunogenic, both with regard to antibody production and to T cell proliferation. However, T cells purified from mice that were immunized with a mixture of self and nonself cytochrome C subsequently proliferated to isolated self cytochrome C. Similar results have been obtained using snRNP.[27] Such results may lead to a process involving APC that simultaneously presents self and nonself peptides, resulting in termination of tolerance to self (see below). This situation may in fact be relevant to the hapten carrier model, as described above, involving LT-ag expression and subsequent binding to host cell nucleosomes.

Analogous to activating autoimmune B cells and T cells by immunization with self and nonself cytochrome C or snRNP, activation of autoimmune nucleosome-specific B cells and T cells could be initiated by linked presentation of nonself LT-ag and self histones, which may be achieved if APCs process and present LT-ag nucleosome complex–derived peptides (Fig. 1). LT-ag-specific T cells respond antigen selectively to presented LT-ag-derived peptides, proliferate, and secrete interleukins. Subsequently, anergic or nonresponsive autoimmune histone-specific T cells are assumed to undergo antigen nonselective cell division in response to interleukins secreted by responder T cells, particularly IL-2 (Fig. 1), and subsequently proliferate antigen selectively in response to presented histone peptides (Fig. 1). In harmony with this model, we have recently demonstrated that T cells from normal individuals stimulated in vitro with nucleosome LT-ag complexes, but not with histones or nucleosomes, subsequently responded to histones and nucleosomes devoid of LT-ag.[20] One explanation for this, in harmony with results obtained by Jenkins,[30] may be that IL-2 by itself terminates experimentally induced T cell anergy by inducing proliferation of the anergic T cells.[30,31]

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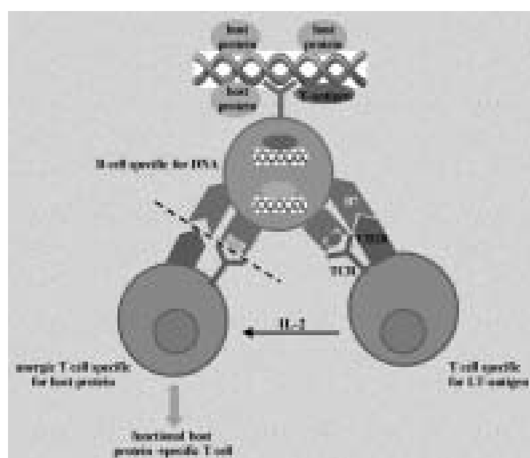

Figure 1. A model based on experimental data for cognate B cell–T cell interaction for the production of anti-DNA antibodies. Nucleosome LT-ag complexes stimulate DNA-specific B cells, which present LT-ag-derived peptides to LT-ag-specific T cells, providing sufficient help for B cells to transform into antibody-producing plasma cells. This pathway is operational if the B cells are acting as true APCs, presenting sufficient costimulatory signals. IL-2 produced by responder LT-ag-specific T cells may influence the responder status of tolerant nucleosome- (histone-) specific T cells, making them operational, provided that B cells copresent histone-derived peptides.

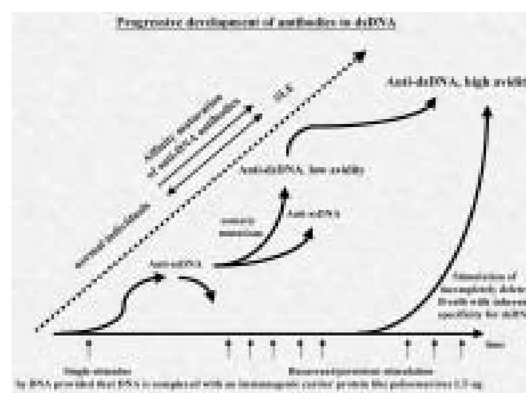

Figure 2. Progressive development of anti-dsDNA antibodies as a consequence of sustained stimulation by DNA. In the left panel, antibodies to ssDNA may gain specificity for dsDNA, along with the progressive immune response, as a consequence of somatic mutations of the antibody variable regions. In the right panel, stimulation is indicated by immunogenic DNA of dsDNA-specific B cells escaping deletion in the bone marrow (see text for details).

LT-ag expressed during polyomavirus infection initiates productive virus replication, on one hand, and binds nucleosomes, on the other.[20,21,32] The latter complex formation may allow presentation of both LT-ag- and histone-derived peptides (Fig. 1). Responder LT-ag-specific T cells may then, through secretion of IL-2, activate autoimmune, histone-specific T cells present in the microenvironment.[30,33] Subsequently, these T cells may clonally expand, provided that histone-derived peptides are presented by APCs in the context of HLA class II and that sufficient costimulatory signals are available[34,35] (Fig. 1). Virtually all human individuals are latently infected with polyomaviruses[2,3] and have therefore expressed LT-ag during primary productive infection. This may explain why all individuals examined so far possess T cells that readily respond to LT-ag complexed with nucleosomes.[20,32,36]

Polyomavirus Infection and Progressive Development of Antibodies to dsDNA

From what we have seen, polyomaviruses, when undergoing productive virus replication in vivo, may initiate processes that may render DNA immunogenic. This insight by itself is not sufficient to explain how antibodies to dsDNA may develop, as most dsDNA-specific B cells may be deleted in the bone marrow.[37] Rather, antibodies to dsDNA may be the result of progressive maturation of B cells as an early anti–single stranded (ss)DNA antibody response evolves along processes demonstrated in Figure 2.

Provided DNA is complexed with immunogenic carrier proteins, an anti-DNA antibody response can be initiated. The early anti-DNA response is characterized by IgM antibodies with specificity for ssDNA (Fig. 2, left panel). Sustained stimulation results in an immunoglobulin class switch to IgG and accumulation of somatically mutated antibody variable regions. If these mutations introduce arginines at discrete positions (e.g., amino acid positions 99-100 of the heavy chain variable region),[38,39] specificity for dsDNA may develop. A B cell clone initially specific for ssDNA may thus gain specificity for dsDNA, and as the immune response progresses, affinity for dsDNA may increase, resulting in high-avidity, potentially pathogenic anti-dsDNA antibodies. This latter antibody population is today regarded as a diagnostic marker for SLE. Alternatively, as outlined in Figure 2 (right panel), B cell clones with inherent specificity for dsDNA may escape deletion[37] and respond to immunogenic DNA. These may then clonally expand directly

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into high-avidity anti-dsDNA antibody-producing cells. Data consistent with this pathway for the generation of anti-dsDNA antibodies have recently been provided.[40]

Conclusion

In summary, polyomavirus infection inherits a new and hitherto less-known consequence linked to virus replication. Normally, these viruses remain silent in the human organism, but occasionally they may reactivate. A prerequisite for this reactivation is expression of the early viral protein LT-ag. As described here, LT-ag may form complexes with host nucleosomes and subsequently initiate both B cell and T cell autoimmunity to nucleosomes. This immune response may, in certain situations, progress into pathogenic immunity, particularly with lupus nephritis as one end result. Thus, virus-induced autoimmunity to nucleosomes may be one operational way to explain the appearance of this form of autoimmunity, typical of SLE. We know that such antibodies to dsDNA deposit in the kidney and initiate nephritis, but the exact mechanisms are highly controversial. Moreover, the exact reasons for vivid reactivation of the polyomaviruses BK and JC in SLE remain obscure. The cause for this reactivation in SLE and the mechanism for anti-DNA antibody-mediated nephritis represent examples of new and challenging problems linked to autoimmunity in SLE that are not solved within the frame of results presented here.

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