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Glomerular expression of large polyomavirus T antigen in binary *tet*-off regulated transgenic mice induces apoptosis, release of chromatin and initiates a lupus-like nephritis☆

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Abstract

Binary tetracycline-regulated polyomavirus large T antigen transgenic mice were generated to study immunological tolerance for nucleosomes. Expression of T antigen resulted in binding of the protein to chromatin, and released T antigen-nucleosome complexes from dying cells maintained anti-dsDNA and anti-nucleosome antibody-production by activating autoimmune nucleosome-specific B cells and CD4+ and CD8+ T antigen specific T cells. Glomerular T antigen expression was observed in these mice. Here, we demonstrate that this expression was linked to glomerular cell apoptosis, release of nucleosomes and association of nucleosomes with glomerulus basement membranes, detected as electron dense structures. Immune electron microscopy (IEM) revealed that these structures were glomerular targets for induced anti-dsDNA and anti-T antigen antibodies. Co-localization IEM demonstrated that *in vivo*-bound auto-antibodies co-localized with experimental monoclonal antibodies to dsDNA and to T antigen. A comparative analysis of glomeruli from nephritic (NZWxNZB)F1 and T antigen expressing transgenic mice revealed deposition of nucleosomes in glomerular capillary and mesangial matrix membranes and binding of anti-nucleosome antibodies in both mice strains. A controlled experimental model that may elucidate the initial events accounting for nucleosome-mediated nephritis has not been available. The transgenic mouse may be important to describe early immunological and cellular events accounting for the enigmatic lupus nephritis.

Keywords: Polyomavirus; Large T antigen; Tetracycline-regulated transgene; Systemic lupus erythematosus; Nephritis; Autoimmunity

1. Introduction

The pathway from production of anti-dsDNA antibodies to glomerular antibody-binding and initiation of lupus nephritis is largely unknown. The individual role of nephritogenic antibodies compared to that of, e.g., cytotoxic T cells in the initial events leading to nephritis is not determined (Bagavant et al., 2004). A consensus has, however, evolved stating that at least sub-populations of anti-dsDNA antibodies have nephritic potential. Experimental and descriptive information opens for two principally different processes resulting in anti-dsDNA antibody-mediated nephritis. Several groups

Abbreviations: DIF, direct immunofluorescence; HE, haematoxylin/eosin; IEM, immune electron microscopy; IIF, indirect immunofluorescence; mAb, monoclonal antibody; MUP, major urinary protein; W/B, (NZWxNZB)F1; PAG-5/10 nm, protein A-5/10 nm gold conjugates; RaM IgG, rabbit-anti-mouse IgG; SC-nucleosomes, spleen cell nucleosomes; TBP, TATA box binding protein; tet, tetracycline; tg, transgenic; TEM, transmission electron microscopy.

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have demonstrated that anti-dsDNA antibodies bind structures located in mesangium and in the glomerulus basement membranes. These structures, recognized as electron dense deposits by transmission electron microscopy (TEM) (Comerford et al., 1968; Dillard et al., 1975; Ben-Bassat et al., 1979), are thought to be chromatin particles released and made accessible for auto-antibodies as a consequence of increased apoptosis in SLE, or of diminished ability to remove apoptotic material (Dieker et al., 2004; Berden, 2003; Berden et al., 2002). Alternatively, anti-dsDNA antibodies may act as a nephritic factor by cross-reacting with renal, non-nucleosomal antigens (Mostoslavsky et al., 2001; Deocharan et al., 2002; Xie et al., 2003; Raz et al., 1993; Termaat et al., 1993). Thus, although there is ample evidence that anti-dsDNA antibodies are central in lupus nephritis, there is still no consensus related to which ligands they *de facto* recognize in the glomerulus.

Recently, we demonstrated data consistent with a model implying trapping of structures recognized by anti-nucleosomal antibodies in glomerular membranes, and binding of anti-DNA and anti-nucleosome antibodies to such membrane-associated structures (Kalaaji et al., 2006a,b). No data consistent with antibody-binding to inherent glomerular membrane constituents or α-actinin was observed in these studies.

If nucleosomes represent glomerular target structures for nephritic auto-antibodies, it is important to understand why chromatin sub-particles are released in lupus nephritis, and why they associate with glomerular capillary and mesangial matrix membranes. It is, therefore, a strong need for models amenable for experimental manipulation to clarify problems linked to initial events of lupus nephritis.

We have during recent years described how productive polyomavirus infection results in activation of dsDNA-specific B cells and nucleosome-specific CD4+ T helper cells. Essentially, we have demonstrated that polyomavirus-encoded large T antigen (T-ag) has the potential to bind chromatin, and thereby to render nucleosomes immunogenic (Moens et al., 1995; Rekvig et al., 1997; Rekvig and Nossent, 2003; Rekvig et al., 2006), in analogy to a hapten-carrier model. To investigate whether constitutive *de novo* expressed T-ag is able to maintain anti-dsDNA antibody production in non-autoimmune mice, we developed a binary transgenic (*tg*) mouse in which expression of SV40 T-ag is controlled by an *Escherichia coli* tetracycline (*tet*) resistance operon (Manickan et al., 2001; Baron et al., 1997); the binary major urinary protein (MUP) tTA/T-ag (*tet*-off) transgenic (*tg*) mouse (Bendiksen et al., 2004). In this *tg* mouse model, post-natal T-ag expression after immunization with T-ag-nucleosome complexes resulted in elevated and remarkably stable titers of anti-T-ag and anti-dsDNA antibodies and activation of T-ag-specific CD4+ and CD8+ T cells (Bendiksen et al., 2004). This system revealed that a *de novo* expressed DNA-binding *quasi* auto-antigen maintained anti-dsDNA antibodies and CD4+/CD8+ T cell activation once initiated by immunization, demonstrating direct impact of a single *in vivo* expressed molecule on sustained autoimmunity to DNA and nucleosomes.

One potentially important aspect of this *tg* model was that the transgene was expressed in glomeruli as well as in the liver (Bendiksen et al., 2004) of binary tTA/T-ag *tg* mice. These mice regularly developed mild proteinuria (unpublished observation). Notably, in *tg* mice, as well as in cell cultures, it has been demonstrated that T-ag has the potential to induce apoptosis (Kolzau et al., 1999; Chen et al., 1998). Furthermore, *de novo* expression of T-ag in glomeruli activates CD8+ T cells that may be able to kill T-ag-expressing glomerular cells (Bendiksen et al., 2004). Thus, glomerular expression of T-ag may result in intra-glomerular cell death by different pathways, and subsequent release of nucleosomes. Such released nucleosomes may carry footprints of this process, as T-ag bind chromatin of expressing cells. Glomerular, extra-cellular membrane-associated nucleosomes may, therefore, be traced by antibodies to nucleosomes and to complexed T-ag. This is a directly testable assumption.

The data presented here reveal that the glomeruli in T-ag-expressing mice undergo changes that are morphologically and immunologically similar to the changes in spontaneous nephritis in lupus-prone (NZBxNZW)F1 mice (Kalaaji et al., 2006a) and in human lupus nephritis (Kalaaji et al., 2007). In glomerular membranes of T-ag-expressing mice, electron dense structures (EDS) are detected that are constituted by nucleosomes and T-ag. In these glomeruli, apoptotic cells are detected. Similar events may be relevant for lupus nephritis.

2. Material and methods

2.1. Antigens and antibodies

Nucleosomes were prepared from murine spleen cells, and T-ag-nucleosome complexes from the constitutively T-ag-expressing mouse fibroblast cell SV-T2, as described in detail elsewhere (Andreassen et al., 1999a,b). Purified nucleosomes derived from spleen and SV-T2 cells, denoted SC-nuc and T-ag-nucleosome complexes, respectively, contained DNA spanning from approximately 200 bp up to several thousand bp (Andreassen et al., 1999a,b). Purification and characterization of polyomavirus SV40 T-ag protein, expressed in recombinant baculovirus, was performed as described (Andreassen et al., 1999a; Bredholt et al., 2001). Calf thymus (CT) dsDNA was obtained from Sigma (St. Louis, USA) and S1 nuclease digested before use.

The following antibodies were used in this study: anti-T-ag monoclonal antibody (mAb) Ab-2 (Oncogene Research Products, Cambridge, MA), anti-TATA box-binding protein (TBP) and anti-histone H1 mAbs (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Alexa Fluor 488-conjugated anti-mouse Fcγ antibodies (Molecular Probes, Inc., Oregon, USA), and peroxidase-conjugated anti-murine IgG antibodies (Sigma). The murine anti-dsDNA mAb 163p77 was obtained from Dr. T Marion (University of Tennessee, Memphis).

2.2. Transgenic and autoimmune mice

The generation and genetical background of *tet*-off regulated T-ag *tg* mice have been described in detail recently (Bendiksen et al., 2004). Binary tTA/T-ag *tg* mice were obtained by crossing the T-ag *tg* mouse with the tTA *tg* mouse (kindly provided by T. Jake Liang, National Institute of Health, Bethesda, MA).