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Immunology

In vivo expression of a single viral DNA-binding protein generates systemic lupus erythematosus-related autoimmunity to double-stranded DNA and histones

UGO MOENS*, OLE-MORTEN SETERNES*, ALLAN WILLIAM HEY*, YNGVE SILSAND*, TERJE TRAAVIK*, BJARNE JOHANSEN*, AND OLE PETTER REKVIG†‡

*Department of Virology, Institute of Medical Biology, University of Tromsø, and †Department of Immunology and Transfusion Medicine, University Hospital of Tromsø, N-9038 Tromsø, Norway

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ABSTRACT **Although the origin of autoimmune antibodies to double-stranded DNA is not known, the variable-region structures of such antibodies indicate that they are produced in response to antigen-selective stimulation. In accordance with this, results from experiments using artificial complexes of DNA and DNA-binding polypeptides for immunizations have indicated that DNA may induce these antibodies. Hence, the immunogenicity of DNA *in vivo* may depend upon other structures or processes that may render DNA immunogenic. We report that *in vivo* expression of a single DNA-binding protein, the polyoma virus T antigen, is sufficient to initiate production of anti-double-stranded DNA and anti-histone antibodies but not a panel of other autoantigens. Expression of a mutant, non-DNA-binding T antigen did result in strong production of antibodies to the T antigen, but only borderline levels of antibodies to DNA and no detectable antibodies to histones. Nonexpressing plasmid DNA containing the complete cDNA sequence for T antigen did not evoke such immune responses, indicating that DNA by itself is not immunogenic *in vivo*. The results represent a conceptual advance in understanding a potential molecular basis for initiation of autoimmunity in systemic lupus erythematosus.**

Antibodies against double-stranded DNA (dsDNA) are highly characteristic of systemic lupus erythematosus (SLE) and important in the pathogenesis of this disease (1, 2). Although the origin of anti-dsDNA antibodies remains controversial (3), results from recent experiments have indicated that DNA itself may be an immunogen (4–8). This has also been indicated by using polyoma virus BK (BKV) or its genomic DNA for induction of such antibodies. Inoculation of animals with BKV has been shown to result in selective production of anti-dsDNA and anti-histone autoantibodies in both rabbits (4, 5) and mice (6). The induced anti-DNA antibodies have recently been shown to be serologically and structurally similar to anti-dsDNA antibodies present in SLE (7). They are clonally selected, somatically mutated, have undergone immunoglobulin class switch, and have arginines in the D regions at positions that favor binding of the antibodies to DNA, indicating that these antibodies are selected and maintained by DNA itself (7). Inoculation of rabbits with protein-free circular but not linear viral dsDNA established a virus infection and induced antibodies to DNA and histones (5). Immunization with BKV DNA–methylated albumin complexes also induced anti-DNA antibodies, but those antibodies were serologically different from autoimmune anti-DNA antibodies in that they bound selectively to viral DNA (5). This association of infection and production of anti-dsDNA antibodies led us to hypothesize that expression of viral DNA-binding protein(s) and/or replication were necessary for induction of antibodies to DNA and histones. According to this hypothesis, viral proteins bind to chromatin in infected cells and thus render host DNA and histones immunogenic. A precedent for the immunogenicity of native, mammalian DNA–peptide complexes has been established (8). An obvious candidate among the polyoma virus encoded DNA-binding proteins is the large tumor antigen (T-ag). This protein is produced *in vivo* during the early phase of virus replication, is required for virus replication, and binds both viral and mammalian dsDNA (9–14).

Since plasmid inoculation has been used successfully as a strategy for induction of antibodies to *in vivo* expressed proteins (15–17), we have applied this technology to test whether T-ag, when expressed *in vivo*, may possess the potential to render DNA and histones immunogenic. This approach may offer several advantages over experimental induction of anti-DNA antibodies by immunizations with preformed artificial complexes of DNA and DNA-binding proteins. The latter experiments have not yielded any information about the origin of molecular mechanism(s) that may render DNA and histones immunogenic *in vivo*. The use of plasmids expressing wild-type DNA binding proteins like polyoma virus T-ag may allow us not only to test whether such a protein may give immunogenic potential to nucleosomes, but also whether an *in vivo* formed complex of nucleosomes and T-ag is processed and presented to the immune system in a way that results in production of autoantibodies to DNA and histones. To test this, groups of five mice were inoculated intramuscularly with purified plasmids containing full-length cDNA for either simian virus 40 (SV40) or BKV T-ag under the control of a eukaryotic promoter supporting constitutive expression of the respective proteins. As demonstrated in this report, autoantibodies to DNA and histones were produced only in mice in which a wild-type, DNA-binding form of the T-ag was expressed.

MATERIALS AND METHODS

Animals. BALB/c mice, 2–3 months old, were obtained from Charles River Breeding Laboratories and housed in the facilities of the Animal Research Department at the Institute of Medical Biology, University of Tromsø.

Plasmids. The plasmids pRSV-BKT and pRSV-L have been described (18, 19). Plasmids pRcCMV-SLT and pRcCMV-SLT_{155S} were constructed by inserting a *Not* I–*Apa* I fragment of the plasmids pSK(−)SVTc or pSK(−)SVTc155TS (14, 20) into the corresponding sites of pRc/CMV (Invitrogen). pRcCMV-BLT and pBS-BLT plasmid constructions: Partial cDNA sequences of BKV T-ag were generated and amplified by reverse transcriptase (RT) PCR on total RNA extracted from BKT-1B cells, which express BKV T-ag (21). The amplification primers

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Abbreviations: dsDNA, double-stranded DNA; T-ag, large tumor antigen; SLE, systemic lupus erythematosus; Th, T-helper cell; MHC, major histocompatibility complex; BKV, polyoma virus BK; SV40, simian virus 40; CT, calf thymus.

‡To whom reprint requests should be addressed.

were BKTT5, 5′-ATGGAATTCAAAAATGGATAAAGTTCTTAACAG-3′, and BKTT3, 5′-TTAGGATCCTGTTGCTTCTTCATCACTGGCA-3′ which contain *Eco*RI and *Bam*HI recognition sites (underlined). The RT-PCR product was ligated in the corresponding sites of pBluescript SK (+/−) (Stratagene). The *Pfl*MI–*Eco*RI fragment was then exchanged with a *Pfl*MI–*Eco*RI fragment of pRSVBKT, which contains the complete C-terminal end of T-ag. This generated pBS-BLT, containing full-length T-ag sequences devoid of their intron. The T-ag sequences were then excised by digestion with *Apa* I and *Not* I and ligated into pRc/CMV to generate pRcCMV-BLT.

***In Vitro* Characterization of Proteins Expressed by the Plasmids.** Immunoprecipitation and immunoperoxidase staining (22) of T-ag were performed on NIH 3T3 cells 2 days after transfection with 4 μg of the appropriate plasmid DNA and 48 μg of Lipofectamine (GIBCO/BRL) per 35-mm culture well. Immunoperoxidase staining of transfected cells was performed as described (22) by using an affinity-purified polyclonal antibody (23) (T81000) to BKV T-ag or the monoclonal antibody Ab-1 (Oncogene Science) to SV40 T-ag. For immunoprecipitation, transfected cells were labeled for 24 h with [^{3}H]leucine and lysed in 200 μl of RIPA buffer (50 mM Tris·HCl, pH 8.0/150 mM NaCl/0.1% Nonidet P-40/0.5% sodium deoxycholate/0.1% SDS) supplemented with 1 mM EDTA/1 mM phenylmethylsulfonyl fluoride/1 μg of leupeptin per ml to inhibit protease activities. T-ag was precipitated from the lysates by using the T81000 or Ab-1 antibody coupled to agarose beads for BKV or SV40 T-ag, respectively. The immunoprecipitates were washed and analyzed by SDS/(4–20% gradient PAGE gel) (Table 1).

Inoculation of Plasmids. Groups of five mice were inoculated with 100 μg of the appropriate plasmid DNA in 100 μl of phosphate-buffered saline (PBS; 0.01 M sodium phosphate/0.15 M NaCl, pH 7.4) per animal in both lumbar regions. Sera were collected prior to the inoculations and every second week over a 10-week observation period.

ELISA. Indirect solid-phase ELISA was performed as described (4–6). Sera were diluted 1/400 in PBS for the assay of IgG antibodies to T-ag and to DNA and 1/100 for the assay of IgG antibodies to histones. Microtiter plates (Nunc) were coated with 5 μg of calf thymus (CT) dsDNA per ml, 1 μg of BKV T-ag as a T-ag–maltose-binding fusion protein (23) per ml, 1 μg of luciferase (Sigma, St. Louis, Mo, USA) per ml, or 1 μg of chicken erythrocyte core histones (24) per ml in PBS. CT dsDNA, T-ag fusion protein, and chicken erythrocyte histones were prepared as described (4, 23, 24). Titers of the induced serum antibodies to the T-ags, DNA, and histones were determined from 2-fold dilution curves. The titers were defined as the reciprocal value of the dilution giving 50% of maximal binding to wells, as determined by individual reference sera (8). The assays were developed with peroxidase-conjugated rabbit anti-mouse Fcγ antibodies (Dako).

Tests for Other Autoantibodies. A hypothetical antigen-nonselective polyclonal B-cell activation initiated by *in vivo* expression of T-ag could theoretically account for the observed production of autoantibodies to DNA and histones. This was examined by testing sera against a panel of other autoantigens not linked to nucleosomes. The presence of autoantibodies to smooth muscles (SMA) and mitochondria (AMA) were examined by a standard indirect immunofluorescence test using tissue sections of mouse kidney and stomach as described (4). For detection of antibodies to thyroid associated antigens—i.e., thyroglobulin (Tg) and thyroid peroxidase (TPO)—as well as to SS-A (Ro), SS-B (La), and RNP, commercially available ELISA tests were used (Shield Diagnostics, Dundee, Scotland). To test for the presence of antibodies to Fcγ [rheumatoid factors (RF)], the standard Latex-RF and the Waaler–Rose tests were performed. The sera were diluted 1/100 in PBS for the ELISA tests to detect antibodies to SS-A, SS-B, RNP, Tg, or TPO. Sera were tested undiluted for the presence of reactivities against SMA and AMA and in the two RF tests.

RESULTS

The characteristics of the T-ag expression plasmids used in these experiments are described in Table 1. T-ag expression from the respective plasmids was verified by *in vitro* transfection of mouse cells. Expressed T-ag was detected by both immunoperoxidase staining of the transfected cells and SDS/PAGE analysis of proteins immunoprecipitated by anti-T-ag antibodies (Table 1). Full-length T-ag expression was observed only from plasmids containing a eukaryotic constitutive promoter: pRSV-BKT, pRcCMV-BLT, pRcCMV-SLT, and pRcCMV-SLT$_{155S}$. No T-ag expression was detected from the pBS-BLT plasmid, which lacks such a promoter.

Three groups of five mice per group each received a single injection of either plasmid DNA expressing wild-type BKV T-ag driven by the Rous sarcoma virus long terminal repeat (pRSV-BKT) or the cytomegalovirus immediate early 1 gene (CMV ie-1) promoter (pRcCMV-BLT) or plasmid DNA expressing wild-type SV40 T-ag under control of the CMV ie-1 promoter (pRcCMV-SLT). The pRSV-BKT plasmid lacks a eukaryotic origin of replication, while the others can replicate in eukaryotic cells (Table 1). Sera were collected over a 10-week period following inoculation with the respective plasmids. Collected sera were independently assayed for IgG antibodies to BKV or SV40 large T-ag, DNA, and histones (Fig. 1 and Table 2). All mice demonstrated a steady increase in antibodies to T-ag after injection of the plasmids, indicating that the protein was also expressed *in vivo*. Likewise, there were parallel increases in serum antibodies to DNA and histones over the same period (Fig. 1 *A* and *B*). After 10 weeks, all mice demonstrated relatively high titers of IgG antibodies to T-ag, DNA, and histones (Table 2), with titers somewhat higher in mice receiving the pRcCMV-BLT plasmid.

Table 1. Properties of the plasmids used to inoculate mice, and demonstration of *in vitro* translation of the full-length, 90-kDa proteins expressed from these plasmids

Plasmid	Eukaryotic promoter	Protein expressed	Expression in cell culture	
			IP*	Size, kDa
pRSV-BKT	RSV LTR†	BKV T/t-ag	Positive	90
pRcCMV-BLT	HCMV ie-1‡	BKV T-ag	Positive	90
pRcCMV-SLT	HCMV ie-1	SV40 T-ag	Positive	90
pBS-BLT	None	None	Negative	Not expressed
pRSV-L	RSV LTR	Luciferase	ND§	ND
pRcCMV-SLT$_{155S}$	HCMV ie-1	SV40 T-ag$_{155S}$¶	Positive	90

*IP, immunoperoxidase staining.
†RSV LTR; Rous sarcoma virus long terminal repeat.
‡HCMV ie-1; human cytomegalovirus immediate-early 1 gene.
§ND, not done.
¶SV40 T-ag$_{155S}$ lacks the sequence-specific, but retains 60% of the weak, nonsequence-specific DNA-binding activity of wild-type T-ag (14, 20).

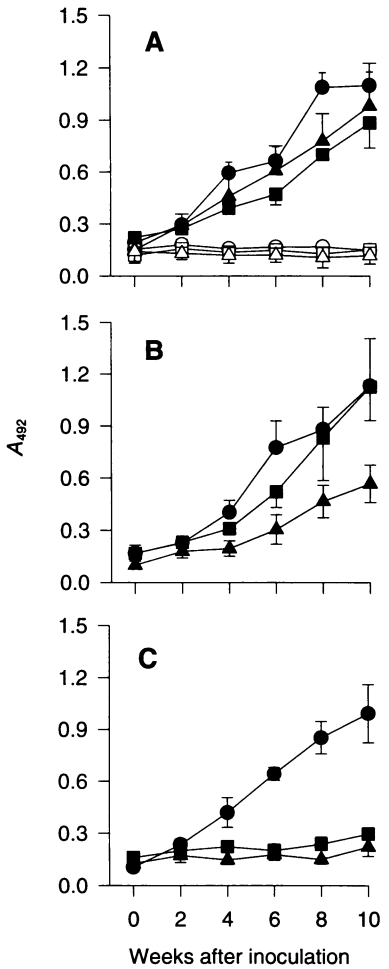

FIG. 1. Immune responses to T-ag (●, ○), DNA (■, □), and histones (▲, △) in groups of mice given a single intramuscular injection of the T-ag-encoding plasmids pRcCMV-BLT (*A*, solid symbols), pBS-BLT (*A*, open symbols), pRcCMV-SLT (*B*), or pRcCMV-SLT$_{155S}$ (*C*). For results from mice injected with pRSV-BKT and pRSV-L, see Table 2. Results are given as mean ± SD of the absorbance at 492 nm representing antibody binding at serum dilutions of 1/400 for antibodies to DNA and the T-ags and 1/100 for antibodies to histones.

These results demonstrate a hitherto undescribed biological activity of both BKV and SV40 wild-type T-ags connected to their potential to induce antibodies, not only to T-ag, but also to DNA and histones.

To better understand how expression of the T-ags acted to initiate these immune responses, different groups of mice were inoculated with one of three control plasmids (Table 1). One group was inoculated with the pBS-BLT plasmid, containing the BKV T-ag cDNA sequence but lacking a eukaryotic promoter. *In vitro* cell cultures transfected with this plasmid produced no detectable T-ag (Table 1). Mice inoculated with pBS-BLT plasmid DNA did not produce antibodies to T-ag, DNA, or histones (Fig. 1*A* and Table 2). These results also demonstrate that plasmid DNA by itself did not induce anti-DNA or anti-histone antibodies in mice receiving T-ag-expressing plasmids. Mice from another group were inoculated with the pRSV-L plasmid. This plasmid contains the complete coding sequence for the firefly luciferase protein (19) driven by a constitutive eukaryotic promoter. Luciferase does not bind DNA according to gel mobility-shift assays (data not shown). These mice did produce antibodies to luciferase but not to T-ag, DNA, or histones (Table 2), demonstrating that expression of a non-self protein by itself did not affect the immunogenicity of DNA or histones. Finally, it was important to establish whether binding of T-ag to DNA was crucial for induction of anti-DNA antibodies. Mice from a third group were therefore inoculated with the pRcCMV-SLT$_{155S}$ plasmid expressing the non-DNA-binding mutant SV40 T-ag$_{155T\rightarrow S}$ (14, 20). This mutant protein retains all the biological functions of the wild-type T-ag, including its ability to transform cells, except that substitution of serine for threonine at amino acid position 155 abolishes sequence-specific binding and substantially reduces the weak, nonspecific DNA-binding capacity of this protein. In response to this plasmid, the mice produced increasing amounts of anti-T-ag antibodies over the observation period. The titers, as well as the timing, of the anti-T-ag antibody responses were similar for these animals and mice receiving plasmids expressing the wild-type SV40 T-ag, indicating no main difference in the expression levels for the two versions of the SV40 T-ag. However, little or no serum antibodies to DNA or histones were observed in these mice (Fig. 1 *C* and Table 2). The very weak production of anti-DNA antibodies (Table 2) after inoculation with the mutant SV40 T-ag-expressing plasmid may have been due to the limited DNA-binding activity of this version of the SV40 T-ag (20). It should be emphasized that we could detect these anti-DNA antibodies only at a serum dilution of 1/100, the lowest dilution tested, and not at the 1/400 screening dilution of the sera. This result demonstrates that T-ag by itself did not act as a nonselective inducer for autoantibody production. This was more directly tested by analyzing the immune sera from mice inoculated with the wild-type T-ag-expressing plasmids—i.e., the pRcCMV-BLT and the pRcCMV-SLT plasmids—for autoantibodies to a panel of eight other autoantigens not related to the nucleosomal complex (see Fig. 2 and *Materials and Methods* for details). No binding to any of these antigens could be detected (Fig. 2). These findings indicate that the induction of antibodies to DNA and histones by the T-ag-expressing plasmids were the results of antigen-selective processes dependent upon the potential for T-ag to bind DNA.

In conclusion, results from the present experiments demonstrate that polyoma virus T-ag may render DNA and histones immunogenic. The linked production of anti-DNA and anti-histone antibodies was confined to mice that received plasmids expressing the wild-type, DNA-binding form of polyoma virus T-ag. Neither plasmid DNA nor non-DNA-binding proteins—i.e., mutant T-ag or luciferase—possessed by themselves the potential to induce these autoimmune responses.

DISCUSSION

To understand the mechanisms of the pathogenic anti-DNA autoantibody response in SLE, it is crucial to determine the nature of the antigen(s) recognized by autoantibody-inducing T helper (Th) cells, whether self, non-self, or both. We have shown that *in vivo* expression of a single non-self DNA-binding protein is sufficient to initiate production of antibodies to DNA and histones. These results indicate that proteins like the polyoma virus T-ag may render autologous DNA and histones immunogenic. Plasmid DNA alone was not sufficient to evoke anti-DNA antibody production. This observation is inconsistent with the hypothesis that plasmid DNA could have non-selectively stimulated B cells to produce antibody (26). The induction of antibodies to DNA and histones was dependent upon the ability of the protein, in this case T-ag, to bind DNA and not on its ability to transform cells. This latter conclusion derives from the fact that there is no difference between wild-type SV40 T-ag and the SV40 T-ag mutated in its DNA-binding domain with respect to the ability of these proteins to transform cells (20). A similar model for stimulating autoreactive B cells has also been proposed by Nemazee *et al.* (27).

Earlier attempts to investigate the role of DNA as the antigen responsible for induction of anti-DNA antibodies have mostly failed. Several main concepts have been suggested for the origin of these antibodies, including nonselective B-cell

Table 2. Characteristics of immune responses to T-ag, CT dsDNA, or histones in BALB/c mice inoculated with various T-ag-expressing and control plasmids

Plasmid	Test antigen, ELISA titer				*Crithidia* test*
	T-ag	CT dsDNA	Histones	Luciferase	
pRSV-BKT	681 ± 40	715 ± 69	557 ± 112	ND	2/5
pRcCMV-BLT	1282 ± 186	871 ± 53	763 ± 87	ND	3/5
pRcCMV-SLT	573 ± 34	360 ± 46	259 ± 52	ND	0/5
pBS-BLT	NB	NB	NB	ND	ND
pRSV-L†	NB	NB	NB	801 ± 96	ND
pRcCMV-SLT_{155S}	442 ± 44	29% ± 2.5‡	NB	ND	ND

BALB/c mice (five individuals in each group) were inoculated with the indicated plasmids, and sera (drawn 10 weeks after the inoculations) were titrated against the indicated test antigens by indirect ELISA. Titers are expressed as the reciprocal value of serum dilutions giving 50% of maximal binding with positive reference sera. The values represent mean titers within each group ± SEM. ND, not done; NB, no binding.

*The *Crithidia luciliae* test is a fluorescence test commonly used for detection of high avidity IgG anti-dsDNA antibodies in SLE (25). The numbers indicate animals that were positive in this test within each group of five animals according to previously established criteria (4).

†The luciferase protein does not bind DNA in gel mobility-shift assays (data not shown).

‡No titer could be determined, as 50% of maximal binding was not reached when using a serum dilution of 1/100, the lowest dilution tested. This dilution gave only 29% of maximal binding.

activation, selective B-cell stimulation (by DNA?), or even stimulation by cross-reactive antigens (for review, see ref. 3). Recent results on the immunogenicity of eukaryotic DNA, however, seem to indicate that DNA possesses the potential to act as a selective inducer of anti-DNA antibody production when combined with a foreign polypeptide (5, 8). Anti-DNA antibodies induced by BKV and BKV dsDNA–methylated bovine serum albumin complexes have recently been shown by us to share characteristics of spontaneously produced, SLE-derived anti-DNA antibodies, with regard both to serological specificities and to molecular structures of the variable regions of the immunoglobulin heavy and light chains (7). The results of such studies have therefore strongly indicated that BKV is able to induce anti-DNA antibodies that (*i*) are highly similar to those derived from SLE and (*ii*) are the result of antigen-selective stimulation of DNA-specific B cells and (*iii*) that DNA may both initiate and sustain the anti-DNA antibody response (6). The structural bases for how DNA achieves the ability to induce these antibodies *in vivo* remains controversial. The present results relate to this problem. In accordance with earlier results, indicating a need for DNA-binding polypeptides to render DNA immunogenic, we provide here strong indications that DNA (or nucleosomes) obtain immunogenic potential as the consequence of *in vivo* complex formation with the non-self T-ag polypeptide. Though not formally proved, the same process may be initiated *in vivo* as a consequence of BKV primary infection and/or reactivations—i.e., expression of the DNA- (or nucleosome-) binding T-ag that may in sum render DNA (or nucleosomes) immunogenic.

As indicated above, the induction of antibody to DNA was dependent upon a functional DNA-binding domain within the expressed T-ag. These results are most consistent with the idea that T-ag bound host-cell DNA in chromatin, thus generating immunogenic complexes. In addition to a requirement for immunoglobulin receptor-dependent antigenic stimulation, B cells also require T-cell help to differentiate to IgG autoantibody-producing cells. Datta and colleagues (28) have previously demonstrated that Th cells specific for nucleosomal proteins, at least one of which is histone, can provide the necessary help in stimulating autoimmune DNA-specific B cells. Although the specificity of Th cells stimulated by the T-ag plasmid immunizations could be for histones, such T cells were more likely specific for the T-ag. The results suggest that cognate T-cell–B-cell interaction was necessary to get efficient induction of anti-DNA antibody since T-ag lacking a functional DNA-binding domain was not effective. In this way, antibodies specific also for dsDNA may be generated by processes similar to that described for development of anti-dsDNA antibodies in autoimmune mice (29–31). The fact that some mice injected with T-ag expression plasmids produced anti-dsDNA antibodies reactive with the dsDNA of the kinetoplast of *C. lucilia* (Table 2), a substrate commonly used to detect anti-dsDNA antibodies in SLE (31), supports this idea.

Antibodies to both DNA and histones but not to other cellular autoantigens were detected in mice immunized with the plasmids encoding wild-type T-ag. These results are also most consistent with an antigen-specific, cognate T-cell–B-cell interaction, rather than noncognate, polyclonal B-cell activation. Subsequent to the expression of plasmid encoded T-ag, MHC class II-positive antigen-presenting cells must have processed the T-ag–nucleosome complexes and presented peptides derived from T-ag to specific Th cells. Once activated, such Th cells could stimulate DNA-specific B cells that presented the appropriately processed T-ag-derived peptides. The exact cellular source of the proposed T-ag–nucleosome complex and the type of antigen-presenting cell that initially presented T-ag-derived peptides to T cells is at present unknown but under investigation.

Another interesting possibility to be investigated within this experimental system is whether DNA-specific B cells once activated could then process and present peptides derived from other DNA-binding nucleoproteins, such as histones. There is certainly precedence for such a process, as demonstrated by Mamula *et al.* (32) and Lin *et al.* (33). Such a process could also explain how autoimmune histone-specific cells detected by Mohan *et al.* (28) were initially generated.

It should be emphasized that the present results may give insight into one possible *in vivo* molecular mechanism that may be operational in initiating production of autoantibodies to dsDNA and histones; yet, it has to be established that proteins encoded by polyoma virus are implicated in these processes during the course of SLE. There are, however, some indications that BKV is reactivated in SLE (ref. 34 and O.P.R., *et al.*, unpublished observation). By following SLE patients for one year, we have observed that in some of them there is a simultaneous production of the virus and antibodies to DNA. In these patients, anti-DNA antibodies were always produced concomitant with active virus replication. These autoantibodies may well be initiated by BKV. However, since anti-dsDNA antibodies were also observed in patients without any sign of virus replication, antigens other than those encoded by polyoma virus genes are also likely to be responsible for initiating anti-DNA antibody production.

We have demonstrated that *in vivo* expression of T-ag may initiate antigen-selective autoimmune responses to DNA and

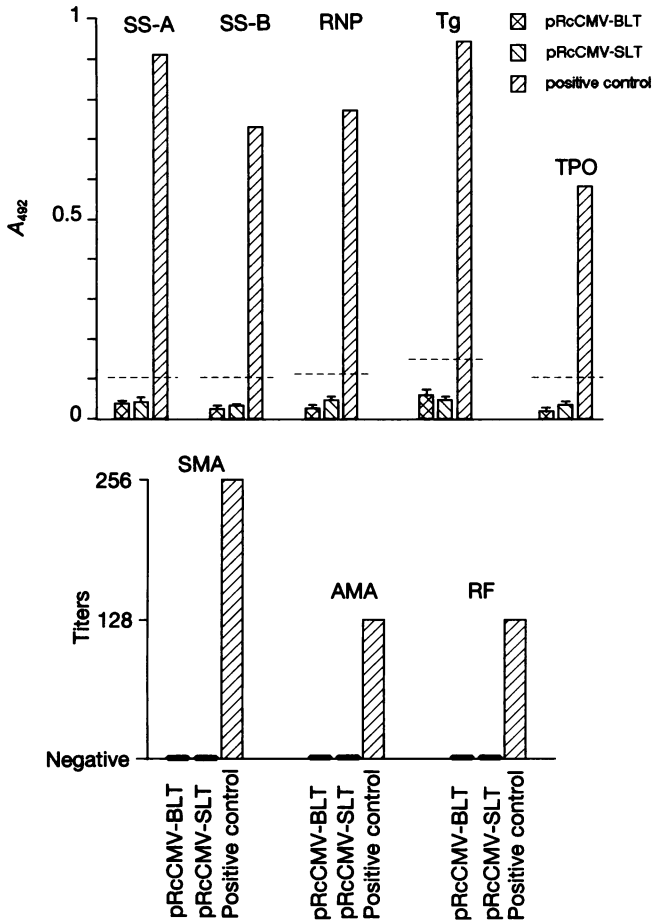

FIG. 2. Tests for the presence of antibodies to antigens not related to the nucleosomal complex in two groups of mice, one inoculated with the pRcCMV-BLT plasmid, expressing the wild-type BKV T-ag, and the other inoculated with the pRcCMV-SLT plasmid, expressing the wild-type SV40 T-ag. (*Upper*) Sera drawn 10 weeks after the inoculations of the plasmids were tested for antibodies against SS-A (Ro), SS-B (La), RNP, thyroglobulin (Tg) and thyroid peroxidase (TPO) by ELISA. (*Lower*) Same sera were tested for antibodies to smooth muscles (SMA) and mitochondrial antigens (AMA) by indirect immunofluorescence tests (IIF), and rheumatoid factors (RF) by the Waaler–Rose hemagglutination test. For the ELISA tests, the sera were tested at a 1/100 dilution in PBS, while for the IIF and Waaler–Rose tests, the sera were tested undiluted and by serial 2-fold dilutions. The horizontal broken lines in *Upper* represent the cut-off levels for the individual tests. For these tests, the data are given as mean ± SD of the absorbance at 492 nm for the five mice within each group. Selected positive controls were included in each individual test.

histones, and future research will elucidate whether cognate interaction of DNA specific B cells and T-ag-specific Th cells subsequently give rise to Th cells specific for self components of nucleosomes. Such self-specific Th cells may eventually account for sustained antibody responses to DNA and histones and, hence, SLE-like autoimmunity.

Antibodies to dsDNA and histones appear to be phenomenologically linked in SLE (35), reflecting the known structural organization of nucleosomes. Taking the present results into consideration, the model system described here may therefore provide an ideal experimental approach to investigate the cellular and molecular mechanisms operating *in vivo* during immune responses to dsDNA and histones.

The results presented in this report may also have important implications beyond the proposed mechanism for induction of anti-DNA and anti-histone antibodies in SLE. As there is a growing interest in the use of expression vectors as vaccines that elicit immune responses to expressed proteins of infectious agents, it may be highly important when developing this strategy to ensure that the aimed immune responses are restricted to the expressed proteins and not accompanied by antibodies to autoantigens induced by complex formation between the expressed proteins and self molecules.

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