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A viral DNA-binding domain elicits anti-DNA antibodies of different specificities

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Abstract

We have previously demonstrated that immunization of normal mice with a defined protein:DNA complex comprising the C-terminal DNA-binding domain of the human papillomavirus E2 protein and its cognate site 35 oligonucleotide, results in high antibody titers against both E2 and its target DNA sequence. Here we show that repeated immunization with the isolated form of the E2 domain also elicits anti-DNA antibodies, but in this case, no preferential binding for a given sequence was observed, indicating that these antibodies have broad specificity for DNA. Taken together our results indicate that this viral protein can induce two classes of anti-DNA antibody responses: one directed against endogenous DNA and other in which anti-site 35-specific antibodies are produced. In both cases, the character of the resulting anti-DNA response seems to be directed by the DNA molecule that the protein binds in vivo. Evaluation of the fine specificity of the antibodies induced by the free and bound states of this single foreign DNA-binding protein would contribute to the understanding of the processes involved in the acquisition of particular DNA specificities by anti-DNA antibodies.

Keywords: Autoantibodies; Anti-DNA antibodies; Transcription factors/gene regulation; Human papillomavirus (HPV)

1. Introduction

Antibodies against native, mammalian DNA are the hallmark of the autoimmune disease systemic lupus erythematosus (SLE). Genetic studies indicate that anti-DNA autoantibodies developed in human disease and animal models are the result of an antigen-driven immune response (Radic and Weigert, 1994; Tillman et al., 1992; van Es et al., 1991). Recurrent germline gene usage and somatic mutations in the V structure of the antibodies strongly suggests that DNA or complexes containing it may provide the antigenic stimulus for DNA-specific B cell activation (Tillman et al., 1992). In the last years, it was demonstrated that anti-DNA antibodies can be experimentally elicited through repeated immunization with protein:DNA complexes in mice not genetically pre-disposed to autoimmune disease (Desai et al., 1993; Desai and Marion, 2000; Krishnan and Marion, 1993; Yan et al., 1995). In those cases, the success of the immunization protocols seemed to rely on the immunogenicity of the heterologous polypeptide used to form the macromolecular complex. In line with these reports, emerging evidences have shown that antibodies against double stranded DNA (dsDNA) can also be elicited during viral

Abbreviations: E2C, C-terminal DNA-binding domain of the E2 transcriptional activator; site 35, double stranded 18 bp oligonucleotide corresponding to one E2 recognition sequence; HPV-16, human papillomavirus strain 16; SLE, systemic lupus erythematosus; T-ag, polyoma virus T-antigen; E2C group, mice immunized with E2C protein; CX group, mice immunized with E2C: site 35 complex; CT-dsDNA, calf thymus dsDNA; CT-ssDNA, calf thymus ssDNA; ARC, the specific operator sequence for the arc repressor; CRE, cyclic AMP-responsive element; VHm, sequence of a mouse immunoglobulin; HPV2.3, an internal DNA sequence of the HPV-16 genome

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infections (Flaegstad et al., 1988). Furthermore, studies have demonstrated that in vivo expression of a single non-self DNA-binding protein, the polyoma virus T-antigen (T-ag), is sufficient to initiate production of antibodies against native DNA and proteins physically linked to it, such as histones and nuclear transcription factors (Moens et al., 1995). A similar pattern of antibody reactivity was observed in SLE patients with frequent polyomavirus reactivation (Rekvig et al., 1997b). Taken together all these results strongly suggest that DNA (or nucleosomes) can obtain immunogenic potential as a consequence of in vivo macromolecular complexes formation with non-self proteins, pointing to a close link between viral infection and autoimmunity to DNA (Rekvig and Nossent, 2003; Van Ghelue et al., 2003).

We have previously reported that immunization of non-autoimmune prone mice with a molecularly defined protein:DNA complex, formed by the C-terminal DNA-binding domain of the E2 protein (E2C) from the human papillomavirus strain 16 (HPV-16) and a 18 bp double stranded oligonucleotide containing one of its recognition sequences (site 35), resulted in a potent antibody response against both the viral protein and its target oligonucleotide. Furthermore, from one of the immunized mice we have obtained and characterized two anti-site 35 IgG monoclonal antibodies (mAb), which recognized the double stranded site 35 oligonucleotide with high affinity and sequence-specificity (Cerutti et al., 2001; Di Pietro et al., 2003). We hypothesized that both the high stability of the E2C:site 35 interaction (Lima and de Prat Gay, 1997) and the potent immunogenicity of the viral protein (Cerutti et al., 2003; Hassen et al., 2001) played a crucial role in the development of these specific anti-oligonucleotide antibodies.

Based on these evidences, we decided to study whether free E2C can also induce an anti-DNA autoantibody response in normal mice, and if so, to compare the profile of the elicited antibodies to those raised against the E2C:site 35 complex. Our results show that increasing antibody titers against mammalian DNA and histones are obtained when the protein alone is used as antigen. In contrast to the sequence specific anti-oligonucleotide response elicited by the site 35-bound E2C, the anti-DNA antibodies induced by free E2C displayed broad specificity for DNA. This would suggest that the specificity of the anti-DNA antibodies induced by E2C immunization depends on the particular stretch of DNA the immunogenic E2 DNA-binding domain binds in vivo. Implications of these phenomena in the context of the current knowledge on the origin of anti-DNA autoantibodies are discussed.

2. Materials and methods

2.1. Antigens

The C-terminal 80-aminoacid DNA-binding domain of the E2 protein from human papillomavirus strain 16 was expressed and purified as previously described (Ferreiro et al., 2000). Histones (type II-S, Sigma Co, USA) were resuspended in 50 mM NaAc pH 5.6, centrifuged and quantified by the Protein Assay ESL kit (Roche, Indiana, USA). The sequence of the A chain of the synthetic oligonucleotides (IDT, USA) used are: site 35: 5′-GTAACCGAAATCGGTTGA-3′; ARC: 5′-ATGATAGAAGCACTCTACTAT-3′; CRE: 5′-AAATTGACGTCATGGTAA-3′; VHm: 5′-GCTACTGGCTACACATTC-3; HPV-2.3: 5′-GCTAACACAGGTAAATCA-3′. Single stranded oligonucleotide concentration determination and annealing were carried out as described (Ferreiro et al., 2000). The E2C:site 35 complex consisted of an equimolar stoichiometric mix of the E2C protein and double stranded site 35 oligonucleotide concentrated in a high ionic strength buffer (25 mM AcNa, 600 mM NaCl, 10 mM DTT, pH 5.6). Calf thymus DNA (Sigma Co, USA) was solubilized in tris buffered saline (TBS) and extracted in phenol/chloroform/isoamyl alcohol (25:24:1) and ethanol precipitated (CT-dsDNA). Single stranded calf thymus DNA (CT-ssDNA) was prepared by heating CT-dsDNA at 95 °C for 5 min followed by rapid chilling in an ice water bath.

2.2. Immunizations

Six-week-old BALB/c mice were immunized i.p. with 16 μg of E2C protein (E2C group) or 25 μg E2C:site 35 complex (CX group) emulsified in the MPL® + TDM Adjuvant System (Sigma Co, USA). All mice were immunized four times at 3-week-intervals and bled 7 days after each immunization. For hybridoma generation, final boosting involved i.p. injection of 20 μg of E2C protein or a freshly prepared protein:DNA 1:1 complex in TBS four days prior to the somatic cell hybridization. Hybridoma cultures were generated following established techniques (Galfré and Milstein, 1981). Briefly, 1×10^8 mouse spleenocytes were fused to 1×10^8 myeloma cells of the line NSO, and the product of the fusion was divided in 96 cultures of 1 ml. Subsequently, between 300 and 400 hybridoma clones were obtained, rendering an average of 3–4 clones per well of culture.

2.3. ELISA assays

ELISA plates were coated either with 0.5 μg per well E2C protein or histones during 1 h at room temperature or with 1 μg per well DNA (CT-ssDNA, CT-dsDNA or double stranded oligonucleotides, see Antigens) over night at 37 °C until evaporation. Sera from lupus patients or immunized mice (diluted in 1% BSA) or undiluted hybridoma culture supernatants where incubated 1 h at room temperature and their reactivity was determined by peroxidase-conjugated polyclonal antibodies to human or murine IgG Fc fragment (Chemicon, USA). The final reaction was developed according to standards procedures. Sera titers were determined by two-fold dilution curves starting at a 1/100 sera dilution. The reciprocal of the serum dilution that gave half of the OD value

of the initial (1/100) dilution of the same serum was taken as the titer.

2.4. Electrophoretic mobility shift assay

Murine RNA was amplified by RT-PCR with degenerate primers for immunoglobulin light chain variable regions. The 400 bp dsDNA product was purified from agarose gels (PCR and Gel Band Purification Kit, Amersham Biosciences) and quantified. An aliquot (0.2 pmol) of dsDNA was incubated with E2C (5 pmol) protein or with E2C plus increasing amounts of site 35 oligonucleotide (0.5–8 pmol). Samples were incubated 25 mM Bis-Tris–HCl pH 7.0, 200 mM NaCl, 1 mM DTT, 8% Glycerol, for 30 min at room temperature. Mixtures were loaded in a 5% polyacrylamide native gel and run in 0.1 M MOPS/imidazol, pH 6.5, at 5 Vcm^{-1}. The bands were visualized by standard ethidium bromide staining and UV transilumination (UVP transiluminator system, GDS8000).

2.5. Data analysis

Statistical analysis was carried out using STATISTICA 4.5 (Statsoft Inc.). *P*-values for comparisons between antibody titers of different groups were estimated by ANOVA and Tukey HSD post-hoc test (individual titer values exceeding $\bar{X} \pm 3$ S.D. were excluded from the analysis). Proportions of anti-DNA positive hybridomas were analyzed by Pearson X^2 test.

3. Results

3.1. E2C elicits autoantibodies towards nuclear antigens in normal mice

The potential of the DNA-binding domain E2C to induce antibodies against mammalian DNA and histones in non-autoimmune prone mice was initially tested by ELISA. Concomitantly with the anti-E2C response, all mice immunized with free E2C (E2C group) developed anti-DNA and anti-histone IgG autoantibodies along the 10-week immunization period (Fig. 1). While the polyclonal response against E2C reached its maximum level after only two boosting immunizations, serum antibody titers against single and double stranded calf thymus DNA and histones continued to increase with successive immunizations (Table 1). In particular, there was a pronounced increase in IgG antibody anti-DNA titers after the fourth immunization, which were significantly higher than those attained after the second and third boosts ($p < 0.0005$, anti-ssDNA and dsDNA reactivity are considered as single statistical group). No cross-reactivity of the induced anti-E2C antibodies with DNA or histones was observed (see below). Similar to what has been observed for the T-ag, these results demonstrates that E2C can effectively elicit the production of autoantibodies against

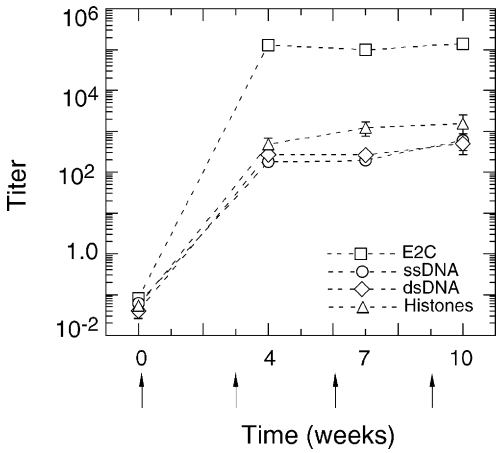

Fig. 1. E2C-terminal DNA-binding domain (E2C) immunization elicits antibodies against mammalian DNA in normal mice. Sera IgG titers against E2C, ssDNA, dsDNA and histones in BALB/c mice immunized with E2C protein are shown. Immunizations were given at 21 days intervals (arrows) and sera were collected 7 days after each immunization.

DNA and histones in normal mice, suggesting that in vivo binding of E2C to endogenous DNA might have occurred.

3.2. Site 35 reduces autoantibody development

As mentioned earlier, immunization of BALB/c mice with E2C:site 35 results in a potent antibody response against the oligonucleotide used to form the protein:DNA complex (mean IgG titer 1000; data not shown) (Cerutti et al., 2001). Thus, once established that E2C alone is capable of inducing both anti-DNA and anti-histone autoantibodies in normal mice, the ability of the site 35-bound E2C to induce such immune response was evaluated. As shown in Table 1, anti-DNA autoantibody titers developed in mice immunized with E2C:site 35 (CX group) remained almost invariable in time, being significantly lower than those achieved in mice of the E2C group after the fourth immunization ($p < 0.0005$ for anti-DNA titers of the CX group at any time point versus the anti-DNA titer of the E2C group after the fourth boost). Comparison of anti-histone antibody titers between these two groups after the fourth immunization gave similar differences ($p < 0.05$). On the other hand, high anti-E2C antibody titers were developed in mice of both groups, indicating that the immunogenicity of the protein was not modified upon site 35 binding (mean IgG sera titer 100,000 for both groups).

These differences in the anti-DNA autoantibody response were further evidenced by analyzing the IgG anti-DNA reactivity of hybridomas generated from spleen cells of mice immunized with E2C or E2C:site 35 complex. Fig. 2 shows the reactivity of the hybridoma culture supernatants against single and double stranded calf thymus DNA. Only hybridomas derived from a mouse immunized with free E2C resulted to be anti-DNA positive, displaying high reactivity against both ssDNA and dsDNA ($p < 0.0005$, E2C versus CX group). On the other side, all screened hybridomas were anti-E2C positive (data not shown), confirming that binding of E2C to its

Table 1
Sera IgG response against nuclear antigens of E2C and E2C:site 35 immunized mice

IgG titer[a]	E2C[b]			CX[b]		
	Second Imm	Third Imm	Fourth Imm	Second Imm	Third Imm	Fourth Imm
ssDNA	181 ± 8	195 ± 17	602 ± 272	202 ± 19	241 ± 37	216 ± 28
dsDNA	269 ± 35	261 ± 14	498 ± 228	256 ± 46	272 ± 59	316 ± 43
Histones	477 ± 212	1225 ± 460	1576 ± 934	517 ± 204	636 ± 64	470 ± 119

[a] Data represent the mean ± S.E. of the IgG antibody titer achieved after each immunization with the indicated immunogen.
[b] Groups of five animals received four boosting immunizations with either 16 μg of free E2C (E2C group) or a stoichiometric mixture of 20 μg E2C:site 35 complex (CX group) emulsified in MPL adjuvant as described in methods. Each dose was administered at 21-day intervals. All immunized mice developed high IgG anti-E2C titers after the second boost (mean titer 100,000).

cognate recognition sequence does not reduce the protein immunogenic potential. Given that in each well of hybridoma culture more than one clone was present, reactivity against both E2C and DNA should not be considered as anti-E2C antibody cross-reactivity. Moreover, 84 out of 94 supernatants displayed reactivity against the E2C antigen only.

3.3. E2C binds native mammalian DNA

In order to test the ability of E2C to bind mouse dsDNA, an electrophoretic mobility shift assay was developed. As shown in Fig. 3, incubation of the viral E2C protein with a 400-bp murine dsDNA fragment produced a notorious delay in the migration of the DNA (compare lanes 1 and 2). Given that no E2 consensus sequences are present in the murine DNA fragments used in the assay, this result indicates that E2C can form a stable complex with mammalian DNA through non-specific interactions, supporting the idea that this protein could potentially bind endogenous DNA in vivo.

We have also postulated that the association of E2C with murine DNA would be strongly impaired when the protein is already bound to its target DNA sequence. In order to test this hypothesis, the migration pattern of the 400 bp dsDNA fragment in presence of the E2C:site 35 complex was analyzed. As observed in the figure, pre-incubation of E2C with its target site 35 oligonucleotide prevents the formation of the E2C:dsDNA complex, which is completely abolished at a 1:1 E2C:site 35 ratio (lanes 3–7). In agreement with the low antibody titers against mammalian DNA displayed by the CX group, this result indicates that once the E2C:site 35 complex is formed, interactions between E2C and murine endogenous DNA are strongly disfavored. This is not unexpected, since E2C has a 10,000-fold higher affinity to site 35 with respect to non-specific DNA oligonucleotides (Ferreiro et al., 2000).

3.4. E2C elicits anti-DNA antibodies of broad specificity

Given the differences in anti-DNA autoantibody titers between mice from the E2C and CX group, we analyzed if immunizations with isolated E2C elicits the production of anti-DNA antibodies with broad specificity for DNA. In this case, the profile of the resulting anti-DNA antibody response would be similar to that observed in SLE, where anti-DNA

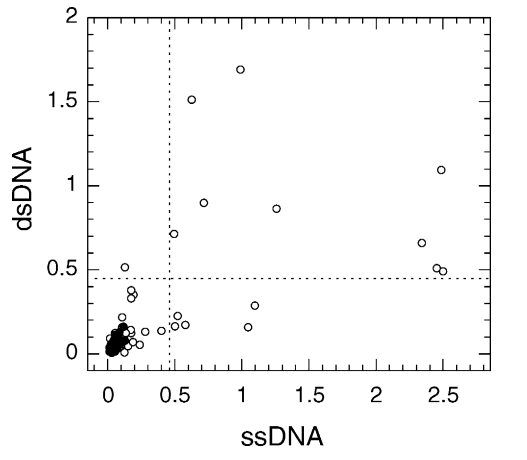

Fig. 2. E2C-terminal DNA-binding domain (E2C) complexation to its recognition sequence precludes anti-DNA antibody production. IgG anti-ssDNA and dsDNA reactivities of hybridoma culture supernatants derived from mice immunized with E2C (open circles) or E2C:site 35 (closed circles) were tested by ELISA. All screened supernatants displayed anti-E2C reactivity (not shown). For the statistical analysis hybridomas with anti-ssDNA and dsDNA reactivities are considered as single statistical group.

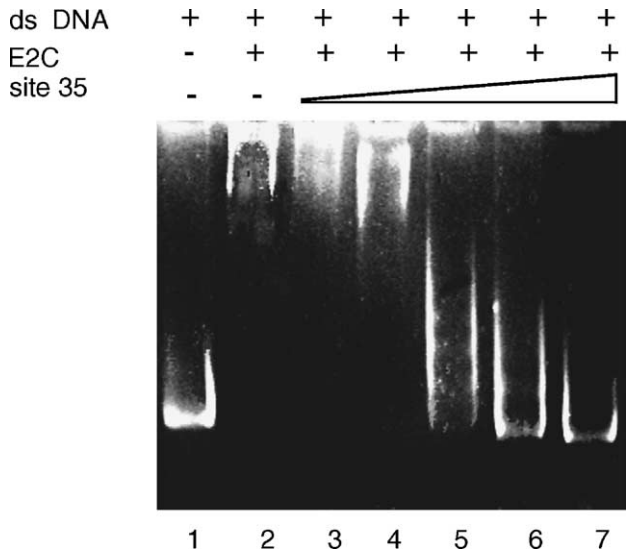

Fig. 3. E2C-terminal DNA-binding domain (E2C) binding to mouse dsDNA is prevented by the cognate site 35 oligonucleotide. 0.2 pmol of 400 bp double stranded murine DNA fragments (free, lane 1) were incubated with 5 pmol E2C protein alone (lane 2) or plus increasing two-fold serial dilutions of site 35 oligonucleotide (0.5–8 pmol, lanes 3–7).

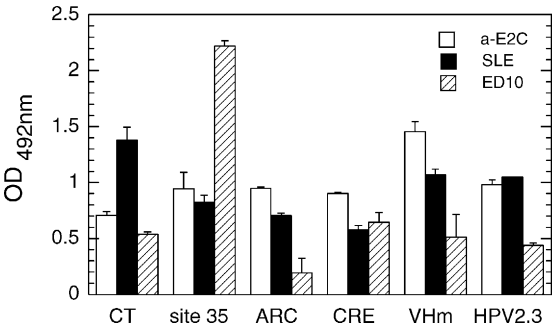

Fig. 4. Anti-DNA antibodies elicited by E2C immunization resemble lupus antibody reactivity. Sera were assayed for IgG reactivity against CT-dsDNA and 18 bp double stranded oligonucleotides of different sequences. a-E2C, pooled sera from E2C immunized mice after the fourth boost. ED10, ascitic fluid of an anti-site 35 IgG mAb (Cerutti et al., 2001). SLE, pooled sera from SLE patients. The oligonucleotides tested for binding were: site 35, one of the HPV-16 E2 recognition sites; ARC, the specific operator sequence for the arc repressor; CRE, cyclic AMP-responsive element; VHm, a random sequence of a mouse immunoglobulin; HPV2.3, an internal DNA sequence of the HPV-16 genome. Sera reactivity of normal mice or healthy humans was subtracted.

antibodies do not have a defined specificity for a given known sequence (Stollar, 1994). On the other hand, preferential binding of E2C to DNA fragments presenting putative E2C binding sites could direct the anti-DNA response towards the recognition of these particular DNA sequences, as occurred when the E2C:site 35 complex is used as antigen. To discriminate between these two scenarios, we assayed the pattern of reactivity of the E2C-immunized mice against a panel of DNA fragments of different sequences and compared it to the pattern of reactivity of sera from SLE patients and the sequence-specific anti-site 35 ED10 mAb. No preferential reactivity against any of the tested DNA sequences was found in the sera of the E2C group (Fig. 4). Moreover, they have broad specificity for DNA, similar to that found in the SLE sera. This result suggests that the stimuli that initiated the production of anti-DNA autoantibodies in mice immunized with free E2C were complexes formed by E2C molecules and fragments of endogenous DNA of no defined sequences.

4. Discussion

We have shown that it is possible to generate monoclonal antibodies against a pre-determined DNA sequence (site 35) (Cerutti et al., 2001). Detailed analysis showed that they bind the oligonucleotide used in the immunization protocol with high sequence specificity and an affinity comparable to that of the natural ligand E2C (Ferreiro et al., 2000). Furthermore, the V region sequence and mutational pattern of the anti-site 35 mAbs indicate that they have arisen during an immune response strongly driven by the antigen, i.e. the E2C:site 35 complex.

Although experimental and descriptive studies on anti-DNA antibodies have contributed to understand the processes involved in their origin and regulation, scarce information linking anti-dsDNA specificity and pathological character is available. Reported data suggest that non-self DNA-binding proteins alone (Moens et al., 1995) or bound to mammalian DNA (Desai et al., 1993; Desai and Marion, 2000) can induce anti-DNA antibodies similar to those developed in SLE. In contrast, anti-DNA antibodies elicited by complexes of bacterial or viral dsDNA with heterologous polypeptides bind poorly to mammalian B DNA, showing high specificity for the dsDNA used as antigen (Cerutti et al., 2001; Gilkeson et al., 1989; Rekvig et al., 1997a). Based on these observations we reasoned that a comparative study of the anti-DNA antibody response induced by a heterologous DNA-binding protein in its free or non-mammalian DNA bound states would help to elucidate the mechanisms leading to activation of B cells with anti-DNA autoreactivity. Immunization of non-autoimmune prone mice with purified E2C protein results in an antibody response against both E2C and nuclear antigens, such as mammalian DNA and histones. Noticeably, E2C immunized mice not only developed IgG anti-ssDNA and dsDNA just after the second boost but showed substantial increases in antibody titers following successive boosting immunizations. This finding agrees with previous reports showing that B cells with ssDNA or dsDNA-specificity may clonally expand if recurrently stimulated with DNA in an immunogenic form (Desai et al., 1993; Desai and Marion, 2000; Krishnan and Marion, 1998; Rekvig et al., 1992; Rekvig and Nossent, 2003). In this regard, it is interesting to note that both the DNA-binding activity of E2C and its high immunogenicity (Cerutti et al., 2003; Hassen et al., 2001) would have played a key role in anti-DNA autoantibody induction.

In the case of the polyoma T-ag, the occurrence of antibodies to DNA and histones was dependent upon its ability to bind DNA and not to its pleiotropic effect on cellular processes (Moens et al., 1997). Expression of a mutant non-DNA binding T-ag protein, which retains the biological function of the wild type T-ag, did result in a strong production of antibodies against it but only borderline levels of antibodies against DNA (Moens et al., 1995). It was also demonstrated that immunization with a fusion protein between the DNA-binding domain of the self-glucocorticoid receptor and a non-self protein, the green fluorescent protein, induce production of anti-DNA antibodies in normal mice (Moens et al., 2002). All these evidences provided strong indications that DNA (or nucleosomes) can obtain immunogenic potential through in vivo association to a non-self DNA-binding protein, in analogy to a hapten-carrier model (see below).

In order to analyze if the anti-DNA autoantibody response raised in mice immunized with E2C alone could have been originated by a similar mechanism, we tested the ability of E2C to bind mammalian DNA in vitro by EMSA. The results show that, by means of non-specific interactions, E2C can form a stable complex with mouse DNA fragments, supporting the idea that this protein has the potential to bind endogenous DNA in vivo. It has been found that E2 is capable of associating with chromatin (Ilves et al., 1999; Lehman and Botchan, 1998; Skiadopoulos and McBride, 1998).

Homologous BPV-1 E2 protein mediates the attachment of the viral genome to the host cell chromatin, coupling host cell cycle with viral genome multiplication and partitioning during latent infection. Although it has been postulated that the N-terminal domain and hinge region of E2 may serve as necessary linkers for tethering viral plasmid to the cell chromatin (Lehman and Botchan, 1998; Skiadopoulos and McBride, 1998), our results indicate that these modules would not be essential for E2 interaction to chromatin DNA at the extracellular milieu. Furthermore, we could also demonstrate that when specific interactions between E2C and site 35 take place, interactions between the protein and murine DNA can no longer be established. This finding is in excellent agreement with our previous work reporting that E2C has an extremely high capacity to discriminate its cognate site 35 oligonucleotide among non-specific DNA sequences of equal length (Ferreiro et al., 2000). All these data constitute solid evidence that, when bound to its specific recognition sequence, E2C has a reduced ability to elicit progressive anti-DNA and anti-histone autoantibodies. These results are reminiscent to those obtained with the non-DNA-binding mutant of T-ag, in which the reduced potential of the protein to generate SLE-like anti-DNA antibodies was a consequence of its incapability to form a physical complex with endogenous DNA.

Global analysis of our results suggests that E2C can induce two classes of anti-DNA antibody responses: one directed against non-particular sequences of endogenous DNA fragments and other in which sequence specific antibodies are produced, i.e. anti-site 35 antibodies. In both cases, the character of the resulting anti-DNA antibody response seems to be mostly determined by the DNA molecule the protein is bound to at the moment of the concerted B and T cell stimulation. In particular, immunization with free E2C directs the anti-DNA immune response towards the development of antibodies with binding specificity similar to those observed in autoimmunity. Alternatively, when the E2C:site 35 complex is used as antigen, a large population of anti-DNA antibodies specifically directed against the unique DNA sequence used to form the protein:DNA complex are developed (Cerutti et al., 2001). We postulate that given the ability of free E2C to bind endogenous DNA, autoreactive DNA-specific B cells would recognize and process the in vivo formed E2C:dsDNA or E2C:nucleosome complexes. Thus, these B cells would present E2C-derived peptides to specific T helper cells present in the microenvironment, conducting to anti-DNA and anti-histone autoantibody development. Conversely, when E2C is already bound to its cognate site 35 oligonucleotide, B-lymphocytes recognizing this complex via its DNA moiety would internalize and process it, directing the anti-DNA antibody response towards production of antibodies with anti-site 35 fine specificity.

Recent examination of the role of viral infections in autoimmunity to DNA and nucleosomes has pointed out that several viruses can be associated with SLE development (Van Ghelue et al., 2003). Two different models for this viral-induced autoimmunity have been proposed, one known as molecular mimicry and the other referred as the hapten-carrier model. Molecular mimicry implies that viral proteins share antigenic determinants similar to host proteins, which lead to autoreactive B and T cell activation. The second mechanism involves lymphocyte activation due to presentation of a self-ligand complexed with a non-self molecule. This last model is proposed to operate in infection with human BK virus, in which viral T-ag may act as carrier of endogenous DNA or nucleosomes, rendering them immunogenic (Rekvig and Nossent, 2003; Van Ghelue et al., 2003). Our present results indicate that this mechanism could also be operational in the human papillomavirus. Further development of a SLE-like experimental model based on HPV E2C immunization would significantly contribute to understand the processes that regulate self-tolerance to DNA.

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References

Cerutti, M.L., Centeno, J.M., Prat Gay, G. de., Goldbaum, F.A., 2003. Antibody response to a viral transcriptional regulator. FEBS Lett. 534, 202–206.

Cerutti, M.L., Centeno, J.M., Goldbaum, F.A., Prat Gay, G., 2001. Generation of sequence specific, high affinity anti-DNA antibodies. J. Biol. Chem. 276, 12769–12773.

Desai, D.D., Krishnan, M.R., Swindle, J.T., Marion, T.N., 1993. Antigen-specific induction of antibodies against native mammalian DNA in nonautoimmune mice. J. Immunol. 151, 1614–1626.

Desai, D.D., Marion, T.N., 2000. Induction of anti-DNA antibody with DNA–peptide complexes. Int. Immunol. 12, 1569–1578.

Di Pietro, S.M., Centeno, J.M., Cerutti, M.L., Lodeiro, F., Ferreiro, D.U., Alonso, L.G., Schwarz, F.P., Goldbaum, F.A., Prat Gay, G., 2003. Specific antibody–DNA interaction: a novel strategy for tight DNA recognition. Biochemistry 42, 6218–6227.

Ferreiro, D.U., Lima, L.M.T.R., Alonso, L.G., Goldbaum, F.A., Prat Gay, G., 2000. Distinctive cognate sequence discrimination, bound DNA conformation and binding modes in the E2 C-terminal domains from prototype human and bovine papillomaviruses. Biochemistry 39, 14692–14701.

Flaegstad, T., Fredriksen, K., Dahl, B., Traavik, T., Rekvig, O.P., 1988. Inoculation with BK virus may break immunological tolerance to

histone and DNA antigens. Proc. Natl. Acad. Sci. U.S.A. 85, 8171–8175.

Galfré, G.A., Milstein, C., 1981. Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol. 73, 3–46.

Gilkeson, G.S., Grudier, J.P., Karounos, D.G., Pisetsky, D.S., 1989. Induction of anti-double stranded DNA antibodies in normal mice by immunization with bacterial DNA. J. Immunol. 142, 1482–1486.

Hassen, E., Chaieb, A., Bouaouina, N., Khairi, H., Remadi, S., Chouchane, L., 2001. Strategies for detection of papillomavirus specific antibodies. Mod. Asp. Immunobiol. 2, 8–12.

Ilves, I., Kivi, S., Ustav, M., 1999. Long-term episomal maintenance of bovine papillomavirus type 1 plasmids is determined by attachment to host chromosomes, which Is mediated by the viral E2 protein and its binding sites. J. Virol. 73, 4404–4412.

Krishnan, M.R., Marion, T.N., 1993. Structural similarity of antibody variable regions from immune and autoimmune anti-DNA antibodies. J. Immunol. 150, 4948–4957.

Krishnan, M.R., Marion, T.N., 1998. Comparison of the frequencies of arginines in heavy chain CDR3 of antibodies expressed in the primary B cell repertoires of autoimmune-prone and normal mice. Scand. J. Immunol. 48, 223–232.

Lehman, C.W., Botchan, M.R., 1998. Segregation of viral plasmids depends on tethering to chromosomes and is regulated by phosphorylation. Proc. Natl. Acad. Sci. U.S.A. 95, 4338–4343.

Lima, L.M., Prat Gay, G., 1997. Conformational changes and stabilization induced by ligand binding in the DNA-binding domain of the E2 protein from human papillomavirus. J. Biol. Chem. 272, 19295–192303.

Moens, U., Mathiesen, I., Ghelue, M.V., Rekvig, O.P., 2002. Green fluorescent protein modified to bind DNA initiates production of anti-DNA antibodies when expressed in vivo. Mol. Immunol. 38, 505–514.

Moens, U., Seternes, O.M., Hey, A.W., Silsand, Y., Traavik, T., Johansen, B., Rekvig, O.P., 1995. In vivo expression of a single viral DNA-binding protein generates systemic lupus erythematosus-related autoimmunity to double-stranded DNA and histones. Proc. Natl. Acad. Sci. U.S.A. 92, 12393–12397.

Moens, U., Seternes, O.M., Johansen, B., Rekvig, O.P., 1997. Mechanisms of transcriptional regulation of cellular genes by SV40 large T- and small T-antigens. Virus Genes 15, 135–154.

Radic, M.Z., Weigert, M., 1994. Genetic and structural evidence for antigen selection of anti-DNA antibodies. Annu. Rev. Immunol. 12, 487–520.

Rekvig, O.P., Fredriksen, K., Brannsether, B., Moens, U., Sundsfjord, A., Traavik, T., 1992. Antibodies to eukaryotic, including autologous, native DNA are produced during BK virus infection, but not after immunization with non-infectious BK DNA. Scand. J. Immunol. 36, 487–495.

Rekvig, O.P., Moens, U., Fredriksen, K., Traavik, T., 1997a. Human polyomavirus BK and immunogenicity of mammalian DNA: a conceptual framework. Methods 11, 44–54.

Rekvig, O.P., Moens, U., Sundsfjord, A., Bredholt, G., Osei, A., Haaheim, H., Traavik, T., Arnesen, E., Haga, H.J., 1997b. Experimental expression in mice and spontaneous expression in human SLE of polyomavirus T-antigen. A molecular basis for induction of antibodies to DNA and eukaryotic transcription factors. J. Clin. Invest. 99, 2045–2054.

Rekvig, O.P., Nossent, J.C., 2003. Anti-double-stranded DNA antibodies, nucleosomes, and systemic lupus erythematosus: a time for new paradigms? Arthritis Rheum. 48, 300–312.

Skiadopoulos, M.H., McBride, A.A., 1998. Bovine papillomavirus type 1 genomes and the E2 transactivator protein are closely associated with mitotic chromatin. J. Virol. 72, 2079–2088.

Stollar, B.D., 1994. Molecular analysis of anti-DNA antibodies. FASEB J. 8, 337–342.

Tillman, D.M., Jou, N.-T., Hill, J., Marion, T.N., 1992. Both IgM and IgG anti-DNA antibodies are the products of clonally selective B cell stimulation in (NZB × NZW) F1 mice. J. Exp. Med. 176, 761–779.

van Es, J.H., Gmelig Meyling, F.H., van de Akker, W.R., Aanstoot, H., Derksen, R.H., Logtenberg, T., 1991. Somatic mutations in the variable regions of a human IgG anti-double-stranded DNA autoantibody suggest a role for antigen in the induction of systemic lupus erythematosus. J. Exp. Med. 173, 461–470.

Van Ghelue, M., Moens, U., Bendiksen, S., Rekvig, O.P., 2003. Autoimmunity to nucleosomes related to viral infection: a focus on hapten-carrier complex formation. J. Autoimmun. 20, 171–182.

Yan, X., Kuhn, S., Traub, P., 1995. A complex intermediate filament protein–DNA: a target for autoantibodies in systemic lupus erythematosus. Chin. Med. Sci. J. 10, 90–92.