Oligonucleotide Linked to Human Gammaglobulin Specifically Diminishes Anti-DNA Antibody Formation in Cultured Lymphoid Cells from Patients with Systemic Lupus Erythematosus

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Abstract

In vitro studies were undertaken to determine whether the level of anti-DNA antibody can be modulated in humans with systemic lupus erythematosus (SLE). DNA fragments of different sizes, i.e., oligonucleotide (N_{20-30}) or oligonucleotide (N_{10-100}), were covalently linked either to human gammaglobulin (HGG) and used as tolerogens or to keyhole limpet hemocyanin and used as immunogens. Experiments were done to determine whether such tolerogens specifically diminish antibodies to denatured DNA, native DNA, or both. PBL were obtained from 87 patients with SLE, 55 of whom spontaneously produced anti-DNA antibodies in vitro. Furthermore, of these 55 test subjects 23 made anti-DNA antibodies in response to antigen challenge in vitro. Exposure of PBL to tolerogenic oligonucleotide-HGG reduced spontaneous antibody formation in 34 of the 55 patients' PBL and abrogated the in vitro-induced response in all instances. The suppression was tolerogen specific. In some SLE patients lymphoid cells were suppressed by both (N_{10-100})-HGG and (N_{20-30})-HGG, while in others lymphoid cells were suppressed by only one. Longitudinal studies of spontaneous antibody production showed that the same tolerogens consistently reduced anti-DNA antibody formation in lymphoid cells of 12 patients on several occasions over a 2-yr interval, but in 8 others the results were either variable or inconsistent. In contrast, tolerogens consistently abrogated the antigen-induced response in all 23 patients' PBL. These results obtained in humans in vitro suggest that the principle of carrier-determined tolerance could be applied as a specific therapy for SLE in vivo.

Introduction

Systemic lupus erythematosus (SLE) is the autoimmune disease par excellence because many autoantibodies to self-antigens, including nucleic acid, are formed (1-3). Whether DNA is an antigenic stimulus in SLE has been a subject of controversy (4, 5), not only because some anti-DNA antibodies cross-react with antigens irrelevant to DNA, but also because other antigens such as cardiolipin and Klebsiella may give rise to antibodies cross-reacting with DNA (6-13). Likewise, the presence of anti-DNA antibodies may or may not correlate

with disease activity (14-17). Despite these paradoxes, anti-nucleic acid antibodies in general, and anti-native DNA (n-DNA)¹ in particular, are still the hallmark of SLE (18, 19). Moreover, antibodies to DNA have been clearly shown to play a role in the mechanism of tissue injury both in murine and human SLE (20, 21). Consequently, specific suppression of anti-DNA antibody by the induction of immunologic tolerance to DNA may have therapeutic value. One way to test this idea is to determine whether conjugates of DNA fragments (oligonucleotides) covalently linked to the self-carrier human gammaglobulin (HGG) specifically modulate anti-DNA antibodies in vitro. The data suggest that both spontaneous and antigen-induced anti-DNA antibodies produced by cultured lymphoid cells from SLE patients can be specifically diminished by oligonucleotides covalently bound to HGG.

Methods

Preparation of antigens and tolerogens. Calf thymus DNA was purchased from Worthington Biochemical Corp. (Freehold, NJ). It was further purified to remove RNA and protein, and then digested with DNase as previously described (22). Total DNA digest was fractionated by chromatography on Ultragel (Pharmacia Fine Chemicals, Piscataway, NJ) as seen in Fig. 1, and fractions were collected. Two sizes of DNA fragments containing either 10-100 (N_{10-100}) or 20-30 (N_{20-30}) bp were obtained. The size of the fragments was determined by PAGE. These oligonucleotide fragments were linked to a protein carrier using glutaraldehyde as a linking agent in a two-step procedure as previously described (23). Two types of immunogens were prepared: total DNA digest (N_{10-100}) and oligonucleotides (N_{20-30}) linked to keyhole limpet hemocyanin (KLH). Likewise, two types of tolerogens were prepared: (N_{10-100}) and (N_{20-30}) linked to HGG as previously described (22). HGG suitable for intravenous therapy was obtained either from Immuno AG or Sandoz Ltd. (Basel, Switzerland) (Sandoglobulin). For specificity controls, we used similar DNA fragments and HGG that were not covalently bound together. In addition, in some instances we used tetranucleoside linked to HGG, prepared as previously described (24), or the immunogen (oligonucleotide) linked to KLH.

Patient population. 87 people with SLE were studied. 79 were patients of Dr. A. Steinberg at the Arthritis Branch of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (Bethesda, MD) and 8 were from the Children's Hospital in Boston. All SLE patients satisfied the diagnostic criteria of the American Rheumatism Association (25). Clinical activity was assessed by the physicians on the basis of signs and symptoms (rash, serositis, arthritis, central nervous system disease, and renal disease) at the time of blood drawing. No attempt was made to separate patients into subsets of

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1. Abbreviations used in this paper: CM, a tissue culture medium containing RPMI 1640 supplemented with 10% fetal bovine serum, 25 mM Hepes buffer, 200 mM glutamine, 1% penicillin/streptomycin, and 5×10^{-5} M mercaptoethanol; d-DNA, denatured DNA; HGG, human gammaglobulin; KLH, keyhole limpet hemocyanin; n-DNA, native DNA.

Figure 1. Chromatography on ultragel of total DNA digest to obtain oligonucleotides. Ultragel fractions (16 drops each) 43–120 represent the total DNA digest (N_{10-100}). Fractions 80–101 represent oligonucleotide (N_{20-30}). — —, IgG; · · ·, a mononucleotide (both used as markers).

clinical activity. Patients lacking these symptoms or signs were categorized as inactive. 65% were active and 35% inactive. 90% of patients with active disease and 75% of patients with inactive disease were receiving corticosteroids. The dose of prednisone never exceeded 1 mg/kg. None of the patients was receiving immunosuppressive drugs such as cytoxan or azathioprine. Corticosteroid was not given on the day blood was drawn. In addition, 24 h elapsed between blood withdrawal, washing, and adding tolerogen to culture medium.

Cultured lymphoid cells from SLE patients. Peripheral blood lymphoid cells were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals) density centrifugation. Cell suspensions were cultured at a final concentration of 5×10^6 cells/ml in flat-bottom microtiter plates (Becton-Dickinson Co., Falcon Labware Div., Rutherford, NJ). 1×10^6 cells were used per culture because this was shown to give an optimum immune response to 1 μ g of antigen (23). The tissue culture medium was RPMI 1640 supplemented with 10% fetal bovine serum, 25 mM Hepes buffer, 200 mM glutamine, 1% penicillin/streptomycin, and 5×10^{-5} M mercaptoethanol. This preparation is referred to hereafter as CM. Cultures were set up in triplicate and maintained at 37°C in a humid atmosphere of 5% CO_2 in air.

Experimental protocol. Tolerogen and antigen solutions were prepared by diluting stock of either (N_{10-100}) conjugate or (N_{20-30}) conjugate in 0.05 M phosphate buffer, pH 7.4, with CM to give a final concentration of 5–50 μ g/ml. 200 μ l of the final dilutions of these preparations was added to each culture. Tolerogens were added in the first culture. As controls, we used a mixture of unbound DNA fragments or oligonucleotides with the same concentration of HGG (control mixture) or each component alone (i.e., oligonucleotide or HGG). For specificity controls we used either nucleosides linked to HGG or oligonucleotides linked to KLH. We found that a 10–24-h incubation with tolerogens was sufficient to decrease spontaneous antibody formation and that a 2–4-d incubation gave similar results. Therefore, an 18-h incubation was chosen, after which the cells were washed three times and resuspended in fresh medium for 4 d. During this 4-d incubation, antigen was either added as described above, or not added, depending on whether either spontaneous or antigen-induced antibody formation was being measured. The cells were again washed three times and resuspended in fresh medium for 6 d. Cells were harvested and antibody was assayed in the culture supernatant.

Solid-phase RIA. Polyvinylchloride microtiter plates (Dynatech Laboratories, Inc., Arlington, VA) were coated with 0.1% protamine sulfate in distilled water for 90 min, rinsed thoroughly with distilled water, then coated with either 50 μ g/ml n-DNA or 50 μ g/ml heat-denatured DNA (d-DNA). Ovalbumin was used at the same concentration as an irrelevant target antigen. All antigens were allowed to stand at

4°C overnight for maximal coating. The coated plates were rinsed three times with a washing solution of 0.25% fetal bovine serum and 0.05% Tween 20 in PBS, pH 7.4.

A blocking solution of 1% BSA and 3% fetal bovine serum in PBS was then added to all wells for 1 h at room temperature. The plate was again washed three times with washing solution, then samples of the culture supernatants were added and allowed to incubate at 37°C for 90 min. Again the plate was washed three times before being developed with ^{125}I -(Fab')₂ adjusted to 10^6 cpm/ml (100 μ l/well) in the blocking solution above. (Fab')₂ was obtained by pepsin digestion of rabbit anti-human Ig which recognizes both IgG and IgM. In some instances, to detect class-specific antibody, ^{125}I -(Fab')₂ goat anti-human reactive with IgM or IgG (New England Nuclear Boston, MA) were used. Plates were developed for 90 min at room temperature, washed as above with washing buffer, and then dried. The plates were cut with scissors and each well was counted for bound radioactivity in a gamma counter. Antibodies to n-DNA, d-DNA, or ovalbumin secreted into the culture supernatant were assayed in triplicate. Background values determined either by the antigen with CM or the irrelevant antigen ovalbumin were $< 600 \pm 30$ cpm, and were subtracted from the experimental values. The data expressed as cpm/culture are the net geometric means of three separate cultures above background. A two-tailed *t* test was used to analyze the significance of the data. For spontaneous antibody production after preincubation with tolerogen, a 30% reduction (> 2 SD compared with controls) was chosen as significant.

Results

Production of either spontaneous or antigen-induced anti-DNA antibody in vitro. PBL from 87 people with SLE were tested for spontaneous production of anti-DNA antibody in vitro. 63% (55 of 87) formed antibody spontaneously. In 107 experiments from these 55 patients antibody to both n-DNA and d-DNA was produced in 45% of the cases, whereas antibody to either n-DNA or d-DNA alone was formed in 20 and 15% of the cases, respectively. None formed antibody to ovalbumin. The range of the spontaneous response expressed as net cpm/culture ± 2 SD above background) varied as follows: 1–150, 8%; 150–300, 22%; 300–1,000, 22%; 1,000–3,000, 32%; and 3,000–15,000, 16%. The class was examined in 50 experiments. Of those, we found that 17 made IgM only, 21 made both IgM or IgG, and 12 made IgG antibody only.

Previously we had shown that one can elicit anti-DNA antibody in vitro by challenging PBL from SLE patients with DNA fragments covalently linked to KLH (23). All 87 patients' PBL were challenged with antigen in vitro. We found that 23 patients' PBL responded by making anti-DNA antibody. All patients producing antigen-induced antibody also formed spontaneous antibody. In all instances, antibodies to both n-DNA or d-DNA were assayed. Six patients' PBL were tested more than once. Of the 30 experiments from 23 patients, 11 gave a response to n-DNA only, 10 to d-DNA only, and 9 to both antigens as seen in Table I. In 24 cases where the class of the immune response was determined, 12 were IgM, 9 IgG, and 3 both IgM and IgG.

Specific reduction of spontaneous anti-DNA antibody formation by oligonucleotide-HGG tolerogen. Initially, we determined the dose of tolerogen necessary to influence spontaneous anti-DNA antibody formation. Fig. 2 shows two representative experiments obtained from PBL of two SLE patients. In both instances there was a 50% reduction of antibody produced to either n-DNA or d-DNA, induced by a dose of 1 μ g/culture of tolerogen. Neither a lower dose (0.1 μ g) nor higher doses (3 μ g or 10 μ g) appeared to be as effective, sug-

Table I.

No. of experiments	Spontaneous antibody		Antigen-induced antibody responses	
	n-DNA	d-DNA	n-DNA	d-DNA
1	1,773	1,200	3,873	15,366
2	16,725	—	33,543	—
3	261	480	744	2,481
4	60	—	810	—
5	—	279	—	1,593
6	3,312	—	7,398	—
7	2,001	—	6,810	—
8	3,246	—	8,700	—
9	4,188	4,160	10,518	10,509
10	1,526	1,428	2,500	2,280
11	711	—	1,338	—
12	—	417	—	870
13	538	—	1,197	—
14	—	1,980	—	3,856
15	462	—	2,853	—
16	—	1,278	—	2,184
17	—	1,656	—	5,265
18	300	296	900	856
19	189	—	414	—
20	—	256	—	516
21	282	321	777	5,571
22	189	240	654	2,232
23	156	285	633	783
24	330	519	2,478	3,618
25	—	492	—	996
26	—	570	—	1,488
27	—	2,502	—	6,666
28	117	—	957	—
29	—	165	—	510
30	672	—	1,755	—

Each figure represents mean cpm/culture per triplicate above background. Each line illustrates the antigen-induced response above the spontaneous immune response.

gesting that the observed reduction was not due to passive absorption of preformed antibody by tolerogen.

Since two different sizes of DNA fragments were used to construct tolerogens (either $[N_{10-100}]$ -HGG or $[N_{20-30}]$ -HGG), we determined whether either one or both conjugates could diminish anti-DNA spontaneously formed in vitro. The results are summarized in Table II. Antibody was reduced by one or both types of tolerogen in 34 of 55 patients' PBL (60%) that produced anti-DNA spontaneously. The specificity of the reduction of antibody spontaneously produced was examined in a number of experiments. A typical example is shown in Fig. 3. DNA fragments must be covalently linked to the carrier (HGG) for the conjugate to be tolerogenic. In contrast, nucleoside linked to HGG failed to affect the response to d-DNA. The specificity was further studied in a number of instances in which one type of tolerogen (either $[N_{10-100}]$ -HGG or $[N_{20-30}]$ -HGG) diminished the spontaneous response. This was done either by linking the same DNA fragment to an immunogenic carrier protein KLH or by using a DNA fragment of a different size linked to HGG. One example of each is given in Fig. 4.

Figure 2. Diminution of spontaneous anti-DNA antibody production by tolerogen. Percent of inhibition as compared with spontaneous antibody formation (100% control). $\circ$, each line represents the result obtained from one SLE patient; $\bullet$, cultured lymphocytes. Note that at high doses there was practically no inhibition, ruling out a nonspecific binding of preformed antibody as the cause of the reduction of antibody production.

Depending on the type of immune response produced, the formation of antibody to either d-DNA, n-DNA, or both can be affected by tolerogen as seen in Fig. 4. Exp. 1 shows a representative experiment in which the production of anti-d-DNA was diminished by both types of tolerogens, whereas Exp. 2 shows anti-n-DNA reduced by oligonucleotide (N_{20-30}) -HGG. Since some SLE patients' PBL spontaneously produced IgG class antibody to either d-DNA or n-DNA, we examined whether we could diminish this class of antibody. Exp. 3 shows a representative experiment in which (N_{10-100}) -HGG suppressed IgG to both anti-d-DNA and anti-n-DNA. It should be emphasized (as seen in Table II) that either one or both oligonucleotides' gammaglobulin conjugates failed to be tolerogenic in $\sim 40\%$ of the cases. The reason could be either that T and/or B cells of this particular patient's PBL were resistant to tolerance induction, or that specificity of the antinucleic acid antibody produced did not correspond to the size of the DNA fragment used to construct a tolerogen. Therefore, it was critical to test the same tolerogenic conjugate on several occasions for the same patient's PBL.

Longitudinal study for the diminution of spontaneous antibody formation. There is considerable variability in the pro-

Table II. Summary of Data Showing Diminution of Spontaneous Anti-DNA Antibody by Tolerogens

Tolerogens used	No. of experiments
(N_{20-30}) -HGG and (N_{10-100}) -HGG	9
(N_{20-30}) -HGG	11
(N_{10-100}) -HGG	14
Total	34

This table represents the composite results of the effect of tolerogens on the response in all 55 patients producing anti-DNA antibody spontaneously. Thus, in 34 of 55 cases (61%) tolerogens were effective. Reduction of spontaneous anti-DNA antibody formation by tolerogens means $< 30\%$ of controls as indicated in Methods.

Figure 3. Specificity of tolerance induction in vitro. Group 1 represents the spontaneous production of anti-d-DNA antibody (control). Group 2 was preincubated with tolerogen oligonucleotide (N_{10-100})-HGG. Group 3 was similarly preincubated with free oligonucleotide (N_{10-100}) not

covalently bound to HGG (control mixture). Group 4 was preincubated with tetranucleoside (AGCT) linked to HGG. Each bar represents the geometric mean of triplicate cultures $\pm$ SEM.

duction of spontaneous antibody by PBL not only among patients but also within each patient. During a 2-yr interval we had the opportunity to study 20 patients' PBL. The results are summarized in Table III. They fall into two broad categories: in a population of 12 patients' PBL we found that the suppression of anti-DNA antibody formation was both consistent and specific for the type of tolerogens used; on the other hand, in a population of eight SLE patients' PBL there is variability both in the ability of tolerogens to diminish antibody formation and in the formation and specificity of antibody produced.

Suppression of antigen-induced antibody production. Next we determined whether pretreatment with tolerogen would influence the immune response after antigen challenge in

Table III. Longitudinal Study of the Diminution of Spontaneous Anti-DNA Antibody by Tolerogens

No. of patients' PBL	Tolerogens		No. of times tested
	(N ₁₀₋₁₀₀)-HGG	(N ₂₀₋₃₀)-HGG	
Group I			
5	+		5
5	—	+	3
2	+	—	3
Group II			
2	+	+	2
	ND	—	1
	No Ab	No Ab	3
4	—	+	5
	+	—	1
	—	—	3
2	+	—	2
	—	—	5

This table represents the number of times 20 patients' PBL were challenged with the same type of tolerogens during a 2-yr interval. This longitudinal study tested the consistency of tolerance induction by one or the other or both types of tolerogen in the same patient's PBL in the course of the disease. In Group I tolerogens consistently reduced anti-DNA antibody, whereas in Group II they did not. +, preincubation of cultured lymphocytes with tolerogen diminishes spontaneous antibody formation; -, no diminution as compared with control cultures not treated with tolerogen.

Figure 4. Diminution of spontaneous anti-DNA antibody by tolerogens. Three examples of reduction of antibody formation by tolerogen. In the first experiment both tolerogens in contrast to the antigen were tolerogenic. The second and third experiments illustrate that either oligonucleotide (N_{20-30})-HGG or oligonucleotide (N_{10-100})-HGG can be tolerogenic. Each experiment was done with a single SLE patient's cultured lymphocytes. ■, antibody to d-DNA; □, antibody to n-DNA.

vitro. This was done not only because production of spontaneous antibody to DNA is both small and variable, but also to determine whether the tolerogen could influence a booster response elicited by exogenous antigen challenge in vitro. We tested PBL from all 23 patients responding to antigen challenge in vitro. Six patients' PBL were tested more than once. Regardless of the type of tolerogen or immunogen used, we found that the antigen-induced response was abrogated by preincubation with tolerogen in all instances except one. This case was tolerized and immunized by both types of tolerogens and immunogens. One tolerogen suppressed only the response elicited by the corresponding immunogen. Fig. 5 shows that (N_{20-30})-HGG abolished the response to the corresponding immunogen. In contrast, the other type of tolerogen failed to diminish the response to the corresponding immunogen. Thus, tolerance induction to n-DNA was tolerogen specific. In 23 of 30 cases the suppression was 95% and in 7 cases 57-95%. Thus, of the 30 cases 7 were tolerized by (N_{20-30})-HGG before in vitro challenge with oligonucleotide (N_{20-30})-KLH and 18 were tolerized by (N_{10-100})-HGG before challenge with (N_{10-100})-KLH. In four instances, different DNA fragments were used as either tolerogenic or immunogenic conjugates. Thus, three were tolerized by (N_{10-100})-HGG before challenge with (N_{20-30})-KLH and one was tolerized by (N_{20-30})-HGG before challenge with (N_{10-100})-KLH. Fig. 6 illustrates two representative experiments in which the IgM anti-d-DNA and n-DNA were assayed. Pretreatment with (N_{10-100})-HGG abrogated the immune response to the corresponding antigen (N_{10-100})-KLH. In contrast, controls made of a mixture of oligonucleotide together with IgG failed to inhibit the response to the antigen. Both IgM and IgG response to native anti-DNA

Figure 5. Specificity of the suppression of the antigen-induced response in a case tolerized and immunized by both types of compounds. Note that only oligonucleotide (N₂₀₋₃₀)-HGG tolerized the immune response induced by the corresponding antigen.

antibody elicited by (N₁₀₋₁₀₀) linked to KLH can be suppressed by (N₁₀₋₁₀₀)-HGG (Fig. 7).

Finally, in some experiments we observed that PBL preincubated with human gammaglobulin together with oligonucleotide (control mixture) resulted in either a decrease or increase of the immune response elicited by the immunogen. We examined the role of either the carrier (HGG) or the hapten (oligonucleotides) in producing this phenomenon. We found that in 15% of the cases HGG could decrease the response of the immunogen, although the suppression was less profound than the tolerogen (Fig. 8). In contrast, in 6 of 30 cases preincubation with oligonucleotide alone appears to prime the antigen-induced immune response (shown in Fig. 5, Exp. 1 and Fig. 7).

Discussion

The development of an antigen-specific immunotherapy for SLE has been hampered not by the ability to induce tolerance to nucleic acid antigen (24, 26), but by the inability to link fragments of DNA to protein carriers to accommodate the combining site of antinuclear acid antibodies made by the SLE patients (22, 27). We have previously shown that oligonucleotide linked to KLH is immunogenic (23). In this paper we have shown that similar DNA fragments linked to human gammaglobulin are tolerogenic, since either spontaneous or antigen-induced anti-d-DNA or anti-n-DNA can be specifically diminished by preincubation of cultured lymphoid cells with DNA fragments linked to HGG. These results obtained in

humans in vitro extend earlier studies done in experimental animals in vivo in which tolerance was induced to small nucleic acid antigens such as nucleosides or nucleotides (24, 28, 29).

Spontaneous production of antibody to DNA was reduced by preincubation with tolerogen in 60% of the cases. In contrast, antigen-induced antibody production was abrogated in all cultures tested but one. Why is this so? First, the amount of spontaneous antibody formed is relatively small and the nature of the endogenous antigen, if any, that stimulates its production is still ill-defined. Second, since both B and T cells' (30) autoreactivity might account for the small amount of antibody spontaneously formed, polyclonal activation might be more resistant to tolerance induction than that induced by exogenous nucleic acid antigen challenge in vitro in which the specificities of tolerogen and immunogen are similar.

Clearly the diminution of spontaneous antibody to either d-DNA or n-DNA was tolerogen specific. Why the immune response in certain patients' PBL was suppressed by both types of tolerogens whereas others were tolerized by either tolerogen is unclear, since the large DNA fragments (N₁₀₋₁₀₀) contain the small fragments (N₁₀₋₃₀). It is known that SLE patients make antibody that recognizes either small or large DNA fragments (27, 31). It is conceivable that after covalent linkage of oligonucleotides of different sizes to the carrier molecule (HGG) the degree of conjugation and/or the spatial presentation of tolerogens might influence their binding to the receptor on lymphoid cells. Furthermore, there is variability in the specificity

Figure 6. Suppression of antigen-induced anti-DNA antibody. Two examples of the specific suppression of IgM anti-DNA antibody production by tolerogen before challenge with antigen in vitro. Note that in Exp. 1 the control mixture (DNA fragments together with HGG but not linked) had a priming effect on the antigen-induced response. ■, anti-d-DNA; □, anti-n-DNA.

Figure 7. Suppression of IgG anti-n-DNA antibody by tolerogen.

of natural anti-DNA antibody production not only among patients' lymphoid cells but also at different times for the same patients' lymphoid cells. Therefore, it was important to test the same tolerogens in the same patients' PBL in the course of the disease. The results of such a longitudinal study showed that in 12 patients' PBL examined several times, the same tolerogens gave consistent results. Similar results were obtained for diminution of anti-DNA antibody elicited by antigen challenge in vitro. On the other hand, there is a group producing spontaneous antibody in which the results are either variable or inconsistent. Because in SLE the auto-antibodies to DNA are polyclonal (6) the variability of the results in some patients' PBL may also reflect the complexity of this issue.

It should be emphasized that both IgM and IgG antibodies to d-DNA or n-DNA formed either spontaneously or after an antigen-induced response in vitro were suppressed. Because high avidity IgG reacting with n-DNA and fixing complement not only causes tissue damage (32), but also correlates with disease activity (14, 19, 33), the observation that IgG antibody to n-DNA can be reduced may have therapeutic value.

Other workers have proposed different therapeutic strategies to suppress anti-DNA antibodies in PBL of SLE patients in vitro. For example, either nucleic acid antigens or antiidiotypic antibodies to DNA have been covalently linked to immunotoxic drugs in an attempt to delete the clone of B cells producing anti-DNA antibody (34, 35). In murine models other investigators have also shown that nucleoside linked to dGL or DNA fragments linked to poly-d-lysine together with cyclophosphamide can suppress anti-DNA antibodies resulting in clinical improvement of murine lupus (36, 37). In this study, we have not examined the mechanism of tolerance in vitro, but in other human models involving haptens linked to HGG we have shown that not only B cells but also T cells are involved (38, 39). Clearly the tolerogenicity of the conjugate is

determined by the carrier molecule HGG. Oligonucleotides have to be covalently linked to the carrier for the conjugate to be tolerogenic, since the mixture of oligonucleotide and HGG is ineffective. The observation that in some instances preincubation with HGG alone can diminish anti-DNA antibody formation is not surprising in view of the finding that HGG suppresses polyclonal activation in vitro (40) and intravenous administration of HGG may interfere with T-B cell cooperation (41). Thus, even if HGG may nonspecifically downregulate the immune response, this further supports its choice as a tolerogenic carrier molecule.

The spontaneous production of anti-DNA antibody by cultured lymphoid cells from people with SLE provides an in vitro model to test the tolerogenicity of DNA fragments of various sizes linked to HGG. Further experiments are needed to determine whether the specificity of the antibody produced by lymphoid cells in vitro corresponds to that produced in the serum in vivo. This question bears on the issue of whether "generic" or "custom-made" tolerogens have to be constructed for the treatment of the disease.

Whether administration of oligonucleotides linked to HGG will influence the production not only of anti-DNA antibodies but also of other antibodies cross-reactive to this antigen is unknown. The specific suppression of anti-DNA antibodies by the induction of immunologic tolerance to DNA might not only allow examination of the role played by immune complexes containing nucleic acid but also that of immune complexes irrelevant to DNA in the pathogenesis of the disease. In conclusion, these data obtained in humans in vitro together with preliminary data obtained in (NZB × NZW) F1 mice (42) suggest that the principles of tolerance can be applied to specifically modulate undesirable autoimmune responses.

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Figure 8. Effect of the carrier protein HGG alone on the diminution of antigen-induced anti-DNA antibody in vitro. Whereas HGG alone reduced antibody formation induced by antigen challenge in vitro, oligonucleotide alone appears to prime the antigen-induced response.

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