Antibodies to DNA and a Synthetic Polydeoxyribonucleotide Produced by Oligodeoxyribonucleotides

Abstract. *Calf-thymus DNA was degraded into small fragments (oligodeoxyribonucleotides); the fragments were treated with methylated bovine serum albumin, and the complexes so formed were emulsified in complete Freund's adjuvant and injected into rabbits. The serums of the immunized rabbits contained antibodies that reacted with homologous and heterologous unfragmented heat-denatured DNA, and also with a synthetic polydeoxyadenylate-thymidylate. Relatively small DNA fragments (of the order of tetra-hexanucleotide) can thus serve as haptens for the production of DNA antibodies; this finding increases the probability of producing antibodies specific for unique sequences of nucleotides.*

Denatured DNA becomes immunogenic after it has formed a complex with methylated bovine serum albumin (MBSA) as hapten carrier (*1*).

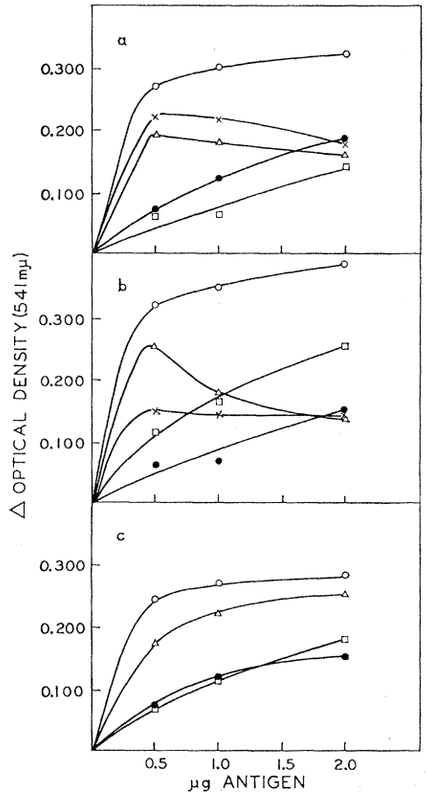

Fig. 1. Analyses of rabbit antiserums for complement-fixing antibodies against homologous and heterologous thermally denatured DNA's. *a*, Antibody of calf-thymus DNA; *b*, antibody to deoxyribonuclease digest of calf-thymus DNA; *c*, antibody to de-purinated calf-thymus DNA. ○———○, Calf-thymus DNA; ×———×, *Brucella abortus*; △———△, *Bacillus subtilis*; □———□, *Diplococcus pneumoniae;* ●———●, monkey-heart cell. The ordinate is the difference between the optical densities of the antiserum control (antiserum + C′) and of the reaction mixture (antiserum + C′ + antigen) and is proportional to the amount of C′-fixing antibody. The volume of antiserum used was 0.1 ml diluted 1:100.

By this procedure precipitating and complement-fixing antibodies to heat-denatured DNA from calf thymus and to heat-denatured T4 bacteriophage DNA were produced. In a somewhat similar way antibodies to DNA have been produced by others (*2*); however, these investigators used only single bases or nucleosides as haptens, and these haptens had to be coupled to the carrier by a covalent bond. The resulting antiserums reacted with DNA because of their specificity for individual bases or nucleosides. In contrast, the antiserums from rabbits injected with the complex of MBSA and denatured calf-thymus DNA contained antibody against sequences of the four common deoxyribonucleotides, as revealed by the fact that each of the deoxyribonucleotides, but not ribonucleotides, deoxyribose, or the bases inhibited the reaction between antiserum and denatured calf-thymus DNA.

The calf-thymus DNA used in these earlier studies was highly polymerized (that is, not fragmented), and it was impossible to decide whether only highly polymerized DNA or also smaller fragments can act as haptens after having formed complexes with MBSA. If relatively small fragments suffice for the production of DNA-specific antibodies, it might be possible to obtain, through the use of homogeneous oligodeoxyribonucleotides, antiserums that are specific for unique sequences of nucleotides.

To determine whether small DNA fragments behave as haptens when complexed with MBSA, calf-thymus DNA was degraded in two ways: (i) by digestion with pancreatic deoxyribonuclease for 24 hours at 37°C (with a 10 : 1 ratio of substrate to enzyme), which results in a mixture of oligodeoxyribonucleotides (*3*), and (ii) by de-purination according to the method of Burton and Petersen (*4*), which yields sequences of pyrimidines. With either procedure, degradation was extensive, and the resulting relatively small fragments of different sizes were not excluded on passage through a Sephadex G-25 filtration gel. (In contrast, heat-denatured DNA used for immunization was completely excluded, an indication that it contained no detectable oligonucleotides.) No attempt was made to fractionate the digests according to size or composition since our primary objective was to determine whether or not the digests contained any fragments that would form complexes with MBSA and act as haptens. However, each digest was analyzed for terminal and total phosphate to obtain an average value for the number of nucleotide residues per fragment. The average was about three, an indication that the digests consisted primarily of relatively short fragments, which is in conformance with the findings of others (*4*, *5*).

A solution of the digest of unheated calf-thymus DNA was mixed with a 1-percent aqueous solution of MBSA (1:1 by weight). Insoluble complexes formed on mixing, but the amount of insoluble material was visibly less than that obtained with undigested heat-denatured DNA. The depurinated preparation did not form insoluble complexes with MBSA. Each mixture was then emulsified with an equal volume of complete Freund's adjuvant for immunization of New Zealand white male rabbits. Each rabbit was given three injections at weekly intervals by way of the foot pad and intramuscular routes. The total amount of DNA-carrier complex (either undigested, digested with deoxyribonuclease, or depurinated) injected into each rabbit was 1 mg. The rabbits were bled by cardiac puncture 10 days after the last injection, and the serums were analyzed for DNA antibodies by complement (C′) fixation (*6*).

All of the serums of rabbits injected with complexes of DNA fragments contained antibodies against homologous denatured calf-thymus DNA (Fig. 1), a clear indication that small fragments of DNA, when complexed to MBSA, act as haptens in the rabbit. The antiserums were also examined for cross-reactions with heterologous denatured DNA preparations. The results of Fig. 1 with representative serums show that antibodies produced against fragments of calf-thymus DNA

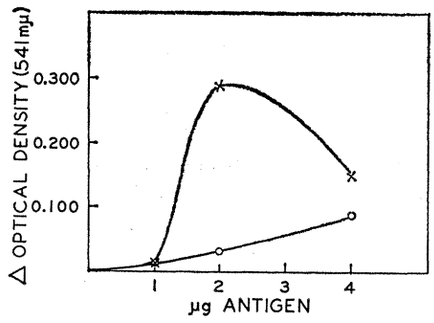

Fig. 2. Analysis of a rabbit antiserum, produced with calf-thymus DNA digested with deoxyribonuclease, for C′-fixing antibodies against a synthetic d-AT polymer: O———O, unheated polymer; ×———×, heated polymer. The volume of antiserum used was 0.1 ml diluted 1:100.

also reacted with heterologous DNA from monkey-heart cells and from several bacterial sources but, as expected, to a lesser extent than with homologous calf-thymus DNA. In addition, these antiserums did not react with calf-thymus DNA digested with deoxyribonuclease and they inhibited the transforming capacities of DNA from *Bacillus subtilis* and from *Diplococcus pneumoniae* (*7*). These findings support the conclusion that DNA-specific antibodies can be elicited by complexes of oligodeoxyribonucleotides with MBSA.

In view of such extensive cross-reactivity and the availability of a synthetic d-AT (polydeoxyadenylate-thymidylate) polymer (consisting of repeating units of adenine- and thymine-containing nucleotides) (*8*), it was of interest to determine whether or not a synthetic polymer also would cross-react.

A solution of the d-AT polymer (40 μg/ml) was boiled at 100°C for 10 minutes to disrupt any hydrogen bonds between adenine and thymine, chilled rapidly in an ice-water bath, and used immediately because of the ease with which hydrogen bonds can form between adenine and thymine. Significant cross-reaction was observed with each antiserum, the extent of the cross-reaction generally paralleling the reaction with homologous calf-thymus DNA. The cross-reaction of an antiserum with both heated and unheated d-AT polymer is shown in Fig. 2. As in the case of natural DNA, the heated preparation was more reactive, and as in the case of natural DNA, this may be attributed to a greater accessibility of the nucleotides for reaction with antibody.

The results show that oligodeoxyribonucleotides can serve as haptens for the production of DNA antibodies. If we take into consideration that the largest fractions occurring in appreciable amounts in a deoxyribonuclease digest of calf-thymus DNA are of the order of hepta-octanucleotides (*4*), and that the largest fraction formed on de-purination with formic acid is a hexanucleotide (*3*), then it would appear that oligodeoxyribonucleotides containing no more than six to eight nucleotides may suffice to serve as hapten when complexed to MBSA. It is unlikely that oligonucleotides containing less than four nucleotides would form stable complexes with MBSA in a physiological medium because the interaction between MBSA and the oligonucleotides is electrostatic in nature. Therefore, the minimum size of oligodeoxyribonucleotide that can serve as a hapten complexed to MBSA can be expected to be of the order of tetra- to hexanucleotide. Stollar *et al.* (*9*) have reported that the immunological specificity of a *Lupus erythematosus* serum is attributable to a pentanucleotide. Therefore, antibodies specific for unique sequences of some five nucleotides may eventually be produced through the use of penta- or hexadeoxyribonucleotides that are homogeneous with respect to size and sequence.

OTTO J. PLESCA
NICHOLAS C. PALCZUK*
WERNER BRAUN, E. CORA-FIGUEROA
Institute of Microbiology, Rutgers University, New Brunswick, New Jersey

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* Present address: Department of Zoology, Rutgers University.

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Polymorphism of Human Lactate Dehydrogenase Isozymes

Abstract. *Of 1200 individuals screened, four had variant erythrocyte lactate dehydrogenases. Three of these, identical electrophoretically to the Mem-1 described by Kraus and Neely, were heterozygous variants involving the* a*-locus. The fourth, involving the* b*-locus, appears to be the first example in man of a homozygous variant.*

Each of the five commonly encountered lactate dehydrogenase (LDH) isozymes is a tetramer containing varying combinations of two different monomers A and B (*1*). Genetic evidence that the synthesis of these two subunits is directed by two different structural genes *a* and *b* was obtained first in mice (*2*), and shortly thereafter in man (*3, 4*). Variants at either the *a*- or *b*-genetic locus were described and pedigrees were presented suggesting autosomal codominant transmission (*3, 4*).

The last and most extensive of these papers, in which eight variant LHD's were discovered among 980 individuals (*4*), raised questions concerning the relative frequency of variants at the two loci and among different populations.

To investigate these questions, erythrocytes from 600 white and 600 Negro patients at various hospitals in the Washington, D.C., area were examined for variant LDH isozymes. Hemolyzates were prepared (*5*) and were subjected to vertical starch-gel electrophoresis in a discontinuous tris-borate buffer system, with a voltage gradient of 12 v/cm² for 3 hours at 4°C. The gels were sliced horizontally, and identification of the LDH isozymes was accomplished by incubation for 1 hour at room temperature in a solution modified from that previously described (*5*) by addition of 4 ml of 0.06*M* sodium cyanide.

Three variants were observed among the 600 Negro patients, a frequency of 0.0050, and one among the 600 white patients, a frequency of 0.0017. Of these three unrelated Negro patients, one, a 65-year-old female, was hospitalized with a cerebrovascular accident probably secondary to atherosclerosis; the second, a 70-year-old female, had hypertensive heart disease, and the third, a 76-year-old male, had chronic lymphocytic leukemia. The pedigree of the second patient revealed six affected