Antibodies Which React with Nucleic Acids

SAM M. BEISER AND BERNARD F. ERLANGER

Department of Microbiology, Columbia University, New York, New York

Summary

During the past 5 years, several procedures for inducing antibodies capable of reacting with nucleic acids have been developed. These include immunization with phage lysates, ribosomes, purines, and pyrimidines or derivatives thereof coupled to proteins and polypeptides, denatured DNA or oligodeoxynucleotides complexed with methylated bovine serum albumin, and Gram-negative bacteria. It is now possible, therefore, to apply immunochemical procedures to the investigation of the chemical and biologic properties of nucleic acids, and some potential applications are discussed.

It is interesting to contemplate, at this Symposium on the 20th anniversary of the founding of the Sloan-Kettering Institute, that the total history of successful immunochemical investigation of nucleic acids encompasses a time span of even shorter duration than the age of this Institute. The presence, in the sera of some patients with systemic lupus erythematosus, of an antibody-like substance which reacted with DNA was demonstrated less than 10 years ago (5, 28, 31, 32), while the 1st unequivocal reports of experimentally induced antibodies capable of reacting with nucleic acids appeared only about 5 years ago. In 1960, Levine et al. (21) succeeded in stimulating the production of anti-DNA antibodies in rabbits immunized with T-even bacteriophage lysates, and Barbu and Panijel (1) demonstrated that injection of ribosomes elicited antibodies specific for RNA. Subsequently, additional techniques for producing antibodies to DNA were developed. These include (a) immunization with purines or pyrimidines coupled to proteins (3, 9, 38) or polypeptides (30); (b) immunization with denatured DNA or polynucleotides adsorbed to methylated bovine serum albumin (26, 27); and (c) hyperimmunization with Gram-negative bacteria (6, 39). We shall limit ourselves to a discussion of anti-DNA antibodies with emphasis on results obtained using conjugated proteins, and a discussion of some potential applications of such antibodies.

Levine et al. (21) provided the 1st unequivocal demonstration of experimentally induced anti-DNA antibodies. The antibodies produced following immunization with T-even bacteriophage lysates reacted only with denatured DNA prepared from these phages and not with native DNA or with denatured DNA from a variety of other sources. Examination of the base composition of the DNA from the T-even phages provided a clue to the basis for this specificity. These DNA preparations contained hydroxymethylcytosine, usually glucosylated, instead of cytosine (18). It was possible to show by hapten inhibition studies (19, 40) that the antibodies were specific for these unique constituents of T-even phage DNA and that the reactivity of the antibodies could be explained on this basis. Because there are differences in the configuration of the glucosylated hydroxymethylcytosines among the T-even phage DNA's, specificity for denatured DNA from each of the phages could be demonstrated (25). The question still remained whether antibodies capable of reacting with other DNA preparations could be obtained. The antibodies to the T-even phage DNA's are inhibited by disaccharides (40) and might also be considered antipolysaccharide antibodies that, fortuitously, react with DNA. However, the results obtained by Levine et al. were extremely important in demonstrating that experimental induction of anti-DNA antibodies was possible and in providing a stimulus for those of us who were becoming somewhat discouraged by a succession of negative results. Levine's work also demonstrated the importance of using denatured DNA when testing for the presence of antibody. This may seem obvious today, but was not so apparent 5 years ago.

A number of protein conjugates containing purines and pyrimidines had been synthesized, e.g., purinyl-6-sulfonyl-BSA, purinyl-β-alanyl-BSA, and orotic acid-BSA, but immunization resulted in antibodies specific only for the protein moiety (S. M. Beiser and B. F. Erlanger, unpublished observations). Bendich and Cohen (7) then called our attention to a compound they had synthesized, 6-trichloromethylpurine. The reactivity of this compound with free amino groups of amino acids suggested that it could be conjugated to proteins. Using this reagent, it was possible to synthesize conjugates containing about 25 purinoyl groupings per molecule of BSA or HSA (3). Immunization of rabbits elicited antibodies which reacted with either purinoyl-BSA or BSA conjugates but which reacted only slightly with BSA or with the purinyl-6-sulfonyl, purinyl-β-alanyl, or the orotic acid conjugates previously prepared. The purinoyl specificity of the antisera was confirmed by hapten inhibition tests. These antisera fixed complement with all denatured DNA preparations tested, but not with native DNA (3). These included preparations from E. coli, B. subtilis, B. natto, H. influenzae, chick embryo, calf thymus, T-even phages, and ϕX-174.

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\textsuperscript{1} The abbreviations used are: BSA, bovine serum albumin; HSA, human serum albumin; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Poly A, polyadenylic acid; Poly C, polycytidylic acid; Poly U, polyuridylic acid; ApC, cytidylyl adenosine; C-BSA, cytidine-BSA; A-BSA, adenosine-BSA; Cpa, adenylyl cytidine; U, uridine.
DNA from the last, present in a single-strand form (34), reacted directly without requiring denaturation.

The reaction between antipurinoyl antibodies and DNA could also be demonstrated by passive cutaneous anaphylaxis and combined immunelectrophoresis and radioautography (4). Precipitation between denatured DNA and this antibody can also occur (2).

Subsequently, antibodies with specificity for a pyrimidine were elicited by immunizing with an acetylmuracil-protein conjugate (38). These, too, reacted with denatured DNA, but also reacted weakly with native DNA.

More recently, a method was developed which made possible the coupling of any riboside or ribotide to proteins (9). This procedure, based upon a reaction used by Khyym (12), for determining terminal nucleotides can be applied not only to monoribosides and monoribotides but also to the coupling of polyribonucleotides to proteins. Conjugates of dinucleotides have already been prepared; larger moieties are being isolated and synthesized for conjugation to proteins.

By coupling the proper nucleoside or nucleotide, antibodies with specificities for adenine, cytosine, guanine, thymine, and uracil have been obtained. These antibodies appear to be quite specific and precipitate with conjugates containing the ribonucleoside or ribonucleotide used for immunization. Very little, if any, precipitate forms with heterologous conjugates or with BSA or HSA (9). The specificities of these antibodies have been confirmed by hapten inhibition.

As was found with the antipurinoyl and antiacetylmuracil antibodies studied previously, the antinucleoside and antinucleotide antibodies react with denatured DNA.

The finer points of the specificities of these antisera are under investigation. Of interest is the finding of a difference in specificity between antibodies induced by adenosine-protein conjugates, and those elicited by adenosine-5'-phosphate coupled to protein (W. Klein, Jr., S. M. Beiser, and B. F. Erlanger, unpublished observations). Whether such differences will also be found with antibodies produced against ADP and ATP-protein conjugates is yet to be determined. It will also be of interest to determine whether such differences in antibody specificity are reflected in the reaction with nucleic acids.

A number of dinucleotides have been coupled to proteins, and some of the properties of the antibodies induced have been studied. Because most of the antisera were anticomplementary, their reactions were investigated using the micro-agar diffusion procedure employed by Lacour et al. (13) to study antibiosome antibodies.

The reaction of anti-dinucleotide sera with Poly A is summarized in Table 1 (E. Nahon, B. F. Erlanger, and S. M. Beiser, manuscript in preparation). This reaction occurs only with antisera to conjugates containing an adenylate group coupled directly to the protein, and not when this moiety is in the terminal position. (No reaction with Poly C or with Poly U was noted with any of these antisera.) This result is surprising in view of the report many years ago by Landsteiner (15) that antibodies elicited by dipeptides coupled to proteins are specific primarily for the terminal amino acid, not the subterminal one. Our results could be explained if ribonuclease in the serum of animals being immunized had cleaved the dinucleotide, leaving only a mononucleotide coupled to the protein to act as antigen.

This would explain the reactions with Poly A. However, if this were the case, there should be no antibodies directed against the terminal nucleotide. Chart 1 shows that anti-ApC (3' -> 5') reacts not only with C-BSA but also with A-BSA. Furthermore, ApC was found to be a better inhibitor than either adenosine or cytidine. Thus, the evidence suggests that the dinucleotide remains conjugated, as such, to the protein, and that the specificity of anti-diribonucleotide antisera is not determined primarily by the terminal component. This is of great potential significance because it may follow, then, that antibodies formed in response to larger oligonucleotides coupled to proteins may recognize the complete coupled moiety rather than just the terminal group.

Using the micro-agar diffusion technic, reactions of antinucleotide antisera with DNA have also been demonstrated. (E. Nahon, B. F. Erlanger, and S. M. Beiser, manuscript in preparation). Anti-ApC and anti-CpA precipitated with DNA prepared from calf thymus, Micrococcus lysodeikticus, Bacillus subtilis, and the pneumococcus. Native DNA did not react, but DNA denatured by heating either in the presence or absence of formaldehyde reacted. It should be noted that DNA from Micrococcus did not react with any of the anti-monoriboside or anti-monoribotide sera tested. Furthermore, only bacterial DNA preparations denatured in the presence of formaldehyde precipitated with antisera to monoribosides or monoribotides. Some typical results are noted in Table 2. The differences in reactivity between anti-diribonucleotide sera and anti-monoriboside or anti-monoribonucleotide sera also indicate that the terminal residue of the former is not removed by ribonuclease in the serum of the immunized animal.

RNA may also react with many of the antibodies studied. The reaction with RNA, however, could not be demonstrated directly, either by complement fixation or by precipitation. However, in the agar diffusion system, yeast RNA inhibited the precipitation with DNA. Sela et al. (30) have reported a direct reaction with RNA; Sela's anti-U precipitated with RNA provided that the RNase normally present in the antisera was neutralized. We could not demonstrate such a reaction with our anti-U sera using the same techniques used by Sela to neutralize RNase. However, Sela prepared his conjugates by coupling uridine-5'-carboxylic acid to polylysine, and it is quite possible

### Table 1

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<thead>
<tr>
<th>Antiserum to**</th>
<th>Reaction with Poly A</th>
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<tr>
<td>ApC (3' -&gt; 5')</td>
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<tr>
<td>ApA (3' -&gt; 5')</td>
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<td>CpA (3' -&gt; 5')</td>
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<td>ApU (3' -&gt; 5')</td>
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<td>UpA (3' -&gt; 5')</td>
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*ApC (3' -> 5'), cytidylyl adenosine; ApA (3' -> 5'), adenylyl adenosine; CpA (3' -> 5'), adenylyl cytidine; ApU (3' -> 5'), uridylyl adenosine; UpA (3' -> 5'), adenylyl uridine; A, adenosine. In the antigen, the base at the right of the abbreviation was coupled to the protein.

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that there is a difference in specificity between the antibodies studied by Sela and those produced in response to the conjugates used in our studies.

Only a small part of the progress made during the past 5 years in the study of antibodies capable of reacting with DXA has been outlined. It may be of interest to review briefly the present status.

Technics have been developed for the experimental production of antibodies reacting with denatured DNA, but, as yet, it has not been possible to produce antibodies that react primarily with native DNA. It may be predicted that this impasse is only temporary, since, among the many sera studied from patients with systemic lupus erythematosus, a rare serum shows a better reaction with native DXA than with denatured DNA (C. Christian, unpublished observations; D. Stollar, unpublished observations). We may speculate, therefore, that it is possible to produce such antibodies but that the proper immunization procedure has yet to be devised.

Except for the work of Levine and his group with the antibodies to the T-even phage DNA, none of the antibodies thus far produced are specific for a particular DNA. The antibodies react with a broad spectrum of denatured DNA preparations, regard-

less of their source or their particular guanine + cytosine content. The antibodies to the T-even phage DNA's, as discussed previously, are directed against a unique determinant and are unusual in this regard. It is not at all certain that immunologic specificity, as it is seen with proteins, will ever be achieved with nucleic acids. Proteins are made up of 20 different components rather than 4 or 5; of greater importance, however, is their capacity to assume stable tertiary configurations, which vary from protein to protein. Denatured DNA apparently can form intrachain hydrogen bonds, but these are apparently formed at random and do not result in unique structures for molecules of different nucleotide sequences. The specificity of the antibody must depend, therefore, on the composition of the nucleic acid and, omitting minor bases, on only 4 components. It is not surprising, then, that antibodies to monoribosides or monoribotides are not capable of distinguishing among DNA molecules. On the other hand, antibodies to known sequences of 3, 4, or 5 nucleotides, when available might be able to do so. Stollar et al. (37) have found that some sera obtained from lupus erythematosus patients have a combining site specific for a unit as large as a pentanucleotide. It would, therefore, appear possible that antibodies specific for moieties larger than dinucleotides could be obtained using suitable conjugates.

Antibodies whose specificity depends on composition are not unique. Heidelberger (10) has found this to be true for antibodies to polysaccharides. With the proper antibody to a polysaccharide component he has even been able to predict the composition of certain carbohydrates—predictions subsequently confirmed by chemical analysis.

It is the natural inclination of most scientists to limit their speculations, at least before large audiences. However, at a symposium such as this, some speculation might be in order. This is not a very strong argument, and we might have been tempted to ignore it had we not recently read a report of a Ciba symposium entitled “Man and His Future.” One of the contributors (41) discusses the biologic future of man for the next 10,000 years. In the light of such long-range prognostication, the following speculation, which may predict only the next 20 years, is analogous to planning tomorrow's experiments.

The possibility should be considered that antibodies to DNA may aid in compositional and structural studies. Perhaps anti-
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Edited by T. M. Sonneborn (35). In it, the possibility of controlling human heredity and of directing specific mutations is discussed. Both Luria and Tatum cite antibodies specific for DNA as a possible means for achieving these ends. The fact that specific methods for altering man's heredity can already be envisaged should, as Luria has pointed out, cause us to begin considering seriously the social implications of man's learning to control his genetic constitution.

Lederberg (17) has coined the term "euphenes" to denote the modification or control of gene expression. Antibodies to DNA may have a role in this area also if we assume that the repressor-operon model proposed by Jacob and Monod (11) to explain genetic regulatory phenomena in bacteria is more widely operative. This model postulates that there are regulator genes, whose product is a repressor substance, and structural genes, which code for the synthesis of enzymes. The repressor, as its name implies, represses the function of a structural gene, or genes, presumably by being able to recognize, and react with, a region known as the operator. An inducer, in some way, renders the repressor inactive and allows the structural gene to be expressed. In short, this is a mechanism, supported by evidence obtained from experiments with bacteria, for turning genes on and off. It has been postulated that a similar mechanism may be operative in cellular differentiation.

Repressors may, as mentioned previously, be specific for a region on the gene, i.e., specific for a particular DNA segment. Therefore, it should be possible to obtain antibodies specific for the operator region of a gene, and such antibodies should act as repressors. To test this possibility, antibodies, or a fragment containing the combining site, must be capable of entering cells. There is a general consensus, which appears to be based on rather meager evidence, that antibodies do not penetrate into bacteria. This should be tested experimentally using protoplasts and antibody fragments. Antibodies with specificities for various purines and pyrimidines appear to be able to penetrate sea urchin embryos, since they have been found to inhibit development at various stages (29). It might follow, then, that under proper conditions, penetration of bacterial cells may be feasible. Even if it is assumed that antibody can enter the bacterium, the specific enzyme deficiency to be sought would still be unknown. With the present rate of progress in molecular genetics, however, the actual nucleotide sequences of some genes and operators, at least in bacteria, should be known soon. It might then be possible experimentally to test the repressor activity of an antibody, since the consequences would be predictable.

Conversely, antibodies might be used to turn on a gene if they were directed against the regulator itself by preventing the formation of the repressor.

It may be possible to test these hypotheses with animal cells in tissue culture without the necessity of knowing which specific enzyme to look for. Such cells frequently lose their capacity to differentiate. If differentiation does depend upon a repressor-operon type mechanism, differentiation might be initiated by exposing cells to various antibodies. For example, bone marrow cells, in tissue culture, lose their ability to differentiate into blood cells and could provide a system suitable for the type of study discussed.

Whatever the cause, or causes, of cancer, all cancer cells are deficient in their regulation of cell division. This could be another...
example of repressor-operon type regulation and might also, ultimately, be amenable to control by antibodies. Of more immediate interest in this area are reports by Sinai et al. (33) and Lacour et al. (14) suggesting that immunization protects against transplanted tumors. Sinai et al. injected purinoyl-HSA and Lacour et al. used ribosomes. Despite the checkered history of previous efforts to immunize against cancer, these reports must be accepted as, at least, encouraging the possibility that a protective immunization procedure may yet be developed.

Plescia et al. have reported that antibodies can be elicited by immunizing with denatured DNA (26) or digests thereof (27), complexed with methylated bovine serum albumin. Despite numerous attempts, immunization with purified DNA alone has not been successful, even with T-even phage DNA with their unique glucosylated hydroxymethylcytosine (L. Levine, unpublished observations). However, the production of antibodies capable of reacting with DXA indicated that there was no inherent, or genetic, block to the antigenicity of DNA. The possibility was considered that deoxyribonucleases normally present in animal sera and tissues destroyed DNA so rapidly that immunogenic fragments never reached immunocompetent cells. If this were the case, DNA might become immunogenic if some means could be found to neutralize the DNase of the recipient animal.

Several years ago, with Dr. Rosenkranz, it was demonstrated that antisera from rabbits immunized with bovine pancreatic DNase reacted not only with the enzyme preparation used for immunization but also with streptococcal and micrococcal DNases. Laufer and Nakase (16), using one of these antisera, found a reaction with the DNase in an insect salivary gland. The wide range of these cross reactions suggested that rabbits immunized with bovine pancreatic DNase might form antibodies capable of neutralizing their own DNase and that DNA might be immunogenic in such animals.

Early results indicate that this is the case. Native calf thymus DNA is immunogenic in rabbits previously immunized with DNase, but, as before, the antibodies reacted with denatured DNA (S. M. Beiser, unpublished observations). It may be, as in the case with lupus sera, that only an occasional rabbit will produce antibodies that react better with native than with denatured DNA, and such a rabbit has not appeared among the few thus far immunized. The antibodies produced are not specific for calf thymus DNA; they react also with many denatured bacterial DNA preparations.

The study of this mode of immunization has just begun, and it is yet to be determined if antibodies specific for a particular DNA might be obtained. It would seem that immunization with DNA alone should be the most probable stimulus for such antibodies if their formation is at all possible.

Obviously, it is of interest to determine whether a similar procedure could be used to obtain antibodies to RNA, i.e., immunization with RNase followed by immunization with RNA. This possibility is being investigated.

The procedure used to make DNA antigenic may have a bearing on current explanations of immunologic “self-recognition.” Most of the theories invoke some mechanism for making cells incapable of responding to the antigen or provide for the total elimination of such antibody-producing cells. Perhaps, based on the results obtained with DNA, self-recognition can be explained by postulating a rapid enzymatic breakdown of the potential antigen before reaching the immunocompetent cell.

In conclusion, it is now established that nucleic acids, although unique in their biologic functions, have at least 2 important characteristics in common with most other biologically active macromolecules: immunogenicity and ability to react with antibodies. The application of the specific, sensitive and selective methods of immunology to the study of the structure and function of nucleic acids has barely begun, and it should lead to an increase in our understanding of, and ability to control, the function of this class of macromolecules.

References


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