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Immunological Recognition of Polynucleotide Structure

F. Lacour, E. Nahon-Merlin and M. Michelson[1]

With 5 Figures

„Not to extract works from works and experiments from experiments . . . But from works and experiments to extract causes and axioms . . . Again from those causes and axioms new works and experiments may arise . . ."

Francis Bacon (1561–1626)

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I. Introduction

Since the discovery of polynucleotide phosphorylase in 1955 and the availability of synthetic polynucleotides, a vast number of physical chemical studies have been pursued with such polymers, in particular by use of optical techniques. (For review see Michelson et al., 1967). It was early shown that

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complementary polynucleotides readily interacted to give DNA type double-stranded helical complexes. Since then, secondary structure in single strands, though less well defined, has been amply explored, as well as formation of triple-stranded complexes under certain conditions. All of these "natural" polymers and polymer complexes adopt a right-handed helical conformation in aqueous solution, and though left-handed structures have been proposed (these do indeed exist in polynucleotides containing L-ribose instead of D-ribose), no rigorous evidence of their existence in natural polynucleotides has been provided. Although the overall geometry is thus right-handed helical, a variety of geometrically distinct forms are known to exist, for example the A, B and C forms of DNA and the A form of double-helical RNA. In addition, chemical and physical studies have shown that synthetic polynucleotide complexes possess different absolute geometries in terms of double helix, and indeed two extreme DNAs, poly dAT from crab, and DNA from *Micrococcus lysodeikticus* (72 % GC) show quite different structures. It is probable that the major factor influencing chemical and physical properties of such helical structures is the relative size of the large and small grooves, governed by the tilting of the planes of the base pairs relative to the helix axis. Indeed it is quite possible that a variable geometry exists within a given DNA molecule as a consequence of relatively long sequences of certain base pairs. However, spectroscopic techniques are unlikely to provide a detailed view of mini portions (e.g. 50 nucleotides long) within a DNA molecule, though this can be done using suitable fluorescent markers (which may themselves alter the local conformation).

In contrast, immunochemical techniques which have already shown a sensitivity sufficient to distinguish single from double-stranded complexes and two-stranded helices from triple-stranded complexes may be extremely useful in such studies. Indeed, recent work using spontaneous antibodies such as those found in systemic lupus erythematosus (SLE) sera, as well as experimentally induced antibodies, has demonstrated immunochemical differences between different double-stranded helical complexes and it is clear that development of this approach will be extremely fruitful.

In terms of antigenic character, a number of possibilities may be considered in polynucleotides. These occur at different levels of sophistication. Thus in single strands, the nature of the bases will play a primary role coupled with that of the sugar phosphate backbone. In double-stranded complexes compared with triple strands, it is rather the external geometry of the structure which should be determinant, though of course some antigenic properties related to the actual bases may still play a role. Of greater interest is the difference between double-stranded structures with different absolute geometries, for example poly rAU (alternating) compared with poly rA·poly rU or poly rG·poly rC compared with poly dG·poly dC. Here it is clear that the helicity itself is an important determinant (number of base pairs per turn of the helix). This review describes preliminary approaches to the problem. Ultimately it should be possible to develop antibodies that are specific not

only for DNA from a given species, but perhaps even for a single gene within a given DNA.

II. Spontaneous Antipolynucleotide Antibodies

We do not propose in this article to analyse various studies already discussed in two general reviews (PLESCIA and BRAUN, 1967; LEVINE and STOLLAR, 1968) and shall present only those observations concerning immunological recognition of the structure of DNA. In addition, discussion will be limited essentially to the most recent results.

A large number of natural antibodies reacting with polynucleotides have been shown to be present in certain pathological sera of mice and men. These sera have been tested with native DNA, or with denatured DNA from various sources, with single-stranded viral DNA, and with synthetic polynucleotides as single chains or in double helical complexes as well as viral RNA. High levels of antibodies reacting with all these polynucleotides have been observed in the sera of patients with SLE and, with the exception of antibodies active against native DNA, in other diseases that generally result in cellular destruction.

1. Antipolydeoxyribonucleotide Antibodies

a) Antibodies reacting with native DNA

The first description of antibodies against polynucleotides was that of the anti-DNA antibody. Such antibodies, in sera of patients, were demonstrated by precipitation, by complement fixation, and by hemagglutination techniques (SELIGMANN, 1957; CEPPELINI et al., 1957; SELIGMANN and MILGROM, 1957; ROBBINS et al., 1957).

From the beginning it was clear that the immune response observed depended largely on the structure of the antigen tested. A systematic study of a large number of sera demonstrated the diversity of anti-DNA antibodies, including diversity of specificity from one serum to another as well as within the serum of the same patient. Although all sera reacted with DNA from any source tested, certain sera reacted with DNA denatured by various treatments and with single-stranded viral DNA from ø X174; others reacted particularly with denatured DNA but also with the same native DNA. Somewhat more rare were sera that gave identical reactions with native and denatured DNA, or a better reaction with native DNA. Given the extremely low concentration of denatured DNA necessary to obtain a reaction with SLE sera, doubt persisted for many years as to the significance of reactions with native DNA, in which a small fraction could have been denatured.

Although exceptional, the existence of antibodies with limited specificity for native DNA shows that the double-helical structure of the molecule could be an inherent part of the antigenic determinant. The role of the native conformation of DNA in these immunological reactions was shown both by the

loss of activity after denaturation of DNA from phage T7 and by the almost complete recovery of this activity when the same denatured DNA was placed under conditions favoring renaturation (ARANA and SELIGMANN, 1967; SELIGMANN and ARANA, 1968). Similarly KOFFLER et al., in 1971, using double helical viral DNA from SV40, showed that the interaction with native DNA could not be due to a small amount of denatured DNA.

Some information on the specificity involved could be obtained by studying cross-reactions between anti-native DNA antibodies and synthetic polynucleotides. With this objective, antigenic studies have been made of polydeoxyribonucleotides, polyribonucleotides and various double helical structures, containing either two polydeoxynucleotides, two polyribonucleotides or the hybrid formed between a polyribonucleotide and a polydeoxyribonucleotide (STOLLAR, 1970; KOFFLER et al., 1971). No reaction of anti-native DNA antibodies with single-stranded polynucleotides such as poly A, poly U, poly C and poly I or with the double helical complexes poly rA·poly rU, poly rI·poly rC or poly rG·poly rC has been observed. In contrast, the anti-native DNA antibodies of SLE patients were found to react with both single-stranded polydeoxyribonucleotides and with double helical polydeoxyribonucleotides. Reaction occurs with alternating poly dAT which has a conformation similar to that of native DNA, as well as with the complexes poly dA·poly dT, poly dG·poly dC and the hybrid polyribo A·poly dT, in which the absolute geometry in terms of double helical structure is different. The results of these studies suggest that, as for globular proteins, the antigenic determinants are controlled to a large extent by ordered structure.

The anti-native DNA antibodies react equally well with DNAs which differ in their composition (DNA from mammalian cells, bacteria or virus) and thus it is unlikely that base sequence determinants are recognized, and specificity is essentially conformation-dependent. Antibodies reacting with native DNA have been described in several other human diseases (KOFFLER et al., 1969; MONIER et al., 1970). However, the level was very low and their specificity has not been studied.

It is interesting to note that antibodies reacting with native DNA have been demonstrated in the sera of New Zealand Black mice (NZB) and of hybrids between NZB and New Zealand White (NZB/W) which spontaneously develop an illness closely resembling human SLE (LAMBERT and DIXON, 1968; STEINBERG et al., 1969). Genetic, immunological and viral factors are involved in the pathogenesis of this disease of the mouse (HOWIE and HELYER, 1968).

b) Antibodies reacting with denatured DNA

Antibodies reacting with denatured DNA which occur frequently in the sera of SLE patients have also been observed in the sera of patients with diseases such as myasthenia gravis (STURGILL et al., 1964), chronic active hepatitis, infectious mononucleosis or rheumatoid arthritis (KOFFLER et al., 1969).

Both the early studies and more recent determination of the specificity of anti-denatured DNA antibodies have shown that the responses vary according to the sera used. The variability of the antigenic determinants is reflected by the difference of susceptibility of the reaction to inhibition by enzymatic products of degradation of DNA of different size, or by apurinic acid. The reactions of several sera with denatured DNA are inhibited by purines, others by pyrimidines or by both. The size of the antigenic determinants is equally variable (see LEVINE and STOLLAR, 1968).

To define the specificity of SLE antibodies reacting with denatured DNA KOFFLER et al., (1971) used polydeoxyribonucleotides as test antigens, or as inhibitors of the reaction. The specificities were multiple: antibodies directed against a determinant common to one or more polydeoxyribonucleotides and to single-stranded DNA, and antibodies with single base specificities were observed. Whatever the specificity of these antibodies, no significant difference appears to exist in the reactivity with DNA from different sources.

2. Antipolyribonucleotide Antibodies

Polyribonucleotides have been used as antigens to recognize and identify other varieties of antibody in the sera of SLE patients and in the sera of New Zealand mice in order to obtain a better understanding of the immunological phenomena associated with the disease.

Antibodies reacting with double-helical viral RNA and with synthetic polynucleotides were effectively demonstrated almost simultaneously in three different laboratories. SCHUR and MONROE (1969) reported the presence of antibodies specifically precipitating double-helical viral RNA (STATOLON), poly rI·poly rC and poly rA·poly rU but not reacting with single-stranded viral RNA, or mammalian RNA. According to these authors these antibodies are specific for double-helical RNA, are different from anti-DNA antibodies, and could be induced by viral RNA. KOFFLER et al. (1969, 1971), using hemagglutination of formalinized red blood cells coated with poly A·poly U, have demonstrated the presence of antibodies reacting with this complex in the sera of SLE patients and in other diseases generally associated with cellular destruction. They also indicated that these antibodies could be inhibited by poly rI·poly rC, and also in certain cases by poly rA and by denatured DNA. They do not exclude the possibility that the formation of antibodies with different specificities might be stimulated by an ubiquitous antigen which could be denatured DNA.

STEINBERG et al. (1969) have shown the existence of antibodies binding specifically with poly rI·poly rC labelled with ^{14}C in the sera of NZB and NZB/W mice as well as in the sera of SLE patients. This reaction can be inhibited by double-helical polyribonucleotide complexes. They showed also that these antibodies are different from anti-native DNA antibodies because the latter are not inhibited by double-helical polyribonucleotides. They later confirmed these results (TALAL et al., 1971) by studies of reciprocal inhibition

and concluded that there were two different populations since DNA inhibited 82% of the reaction with radioactive DNA whereas only 6 % inhibition of the reaction with poly rI·poly rC was obtained.

A study of the specificity of anti-RNA antibodies has been made on SLE sera selected for their capacity to bind a large proportion of radioactive poly rI·poly rC. Comparisons were made of the inhibitory capacities of two double helical viral RNAs, of ribosomal RNA, of tRNA and of two synthetic double helical complexes. The viral RNAs were the most effective inhibitors, RNA from reovirus and RNA from mycophage inhibiting in certain cases 100 % of the reaction with poly rI·poly rC; the complexes poly rI·poly rC and poly rA·poly rU, as well as ribosomal RNA, were less active; and, finally, tRNA from rat liver, from *E. coli* or from yeast showed a rather low capacity for inhibition. While the specificity of these human antibodies varies according to the individual, the specificity of the New Zealand mice sera is more homogeneous. Reovirus RNA is again the best inhibitor of the reaction and smaller quantities than those required for poly rI·poly rC or poly rA·poly rU are sufficient to inhibit 50 % of the reaction with the radioactive complex. This much greater reactivity of double-helical viral RNA indicates that the structure is closer to that of the immunogen which has induced the antibodies. Talal et al. (1971) and Schur and Monroe (1969) suggest that formation of these anti-double-helical RNA antibodies could be the result of a viral infection.

Since the stimulation is unknown in the case of naturally appearing antibodies showing such varied specificities, it is hardly to be hoped that more precise information could be obtained on the particular group or the arrangement of the antigenic groups which induce the antibodies or which serve as recognition points. The existence of antibodies specific for the DNA double helix, single-stranded DNA or double helical RNA nevertheless suggests that different structural determinants are involved. Studies on the specificities of experimentally induced anti-polynucleotide antibodies have reinforced this belief.

III. Experimentally-Induced Antisynthetic-Polynucleotide Complex Antibodies. Role of the Conformation of the Immunogen

1. General Considerations

With the exception of antibodies reacting only with native DNA all the different kinds of anti-polynucleotide antibodies described above can be induced experimentally.

Although in most cases the experimentally induced antibodies react like the natural antibodies with nucleic acids from various sources, it is possible to induce antibodies specific for a particular DNA, for a polydeoxyribonucleotide complex or even to obtain antibodies reacting selectively with certain kinds of natural RNA.

In only two cases have antibodies specific for a given DNA been induced experimentally. These antibodies were obtained by immunization with a lysate of phage T 4 (LEVINE et al., 1960) and by immunization with DNA from phage 2 C adsorbed to methylated bovine serum albumin (MAY-LEVIN et al., 1967). In both these cases the specificity is related to the presence of an abnormal base in the DNA; thus glucosylated hydroxymethyl cytosine replaces cytosine in coli phage T 4 and in phage 2 C, thymine is replaced by 5-hydroxymethyluracil. Such antibodies react solely with the DNA containing the base against which they are directed.

The specificity of an experimentally induced antibody is also associated with a particular base when the usual components of nucleic acids, whether purines (BUTLER et al., 1962) or pyrimidines (TANENBAUM and BEISER, 1963), nucleosides or nucleotides (ERLANGER and BEISER, 1964; SELA et al., 1964), are used as immunogens after conjugation with a protein or with polypeptides. Such antibodies recognize the particular base in various polynucleotides and especially in denatured DNAs. The importance of secondary structure in polynucleotides was recognized early (LEVINE et al., 1960; see LEVINE and STOLLAR, 1968). LACOUR and HAREL (1965) demonstrated that for recognition of adenine in different polynucleotides by antibodies to adenosine — BSA, it was necessary that the base should not be involved in complementary hydrogen bonding.

No specificity for a particular DNA is obtained when dinucleotides or oligonucleotides are conjugated with a carrier protein, when DNA complexed to MBSA, or whole bacteria, are used as immunogens; the antibodies thus induced react with a variety of denatured DNAs (BEISER and ERLANGER, 1966; PLESCIA et al., 1964; HALLORAN and PARKER, 1966; TAN and NATALI, 1970; FORSÉN et al., 1970; CHRISTIAN, 1965; BOROS and OLITZKI, 1967; FAVORSKAYA, 1970).

The large spectrum of cross-reactions with different denatured DNAs is readily explained by the fact that the number of different bases involved in deoxynucleic acids is extremely limited and that the four major bases are composed of only two main classes, the purines and the pyrimidines. The absence of cross-reactions with native DNA has been attributed to the non-accessibility of the antigenic sites, the bases lying in the interior of the double-helical structure.

Antibodies to RNA induced by immunization with ribosomes react with RNAs whatever the origin (BARBU and PANIJEL, 1960, 1961; BIGLEY et al., 1963) but can nevertheless distinguish ribosomal RNA from tRNA (LACOUR et al., 1962). They also differentiate between single-stranded polyribonucleotides and double-stranded polyribonucleotide complexes (NAHON et al., 1965). A structural study of multi-stranded polynucleotides with purified anti-RNA immunoglobulins has been reported by PANIJEL et al. (1966a and b). Thus whether polydeoxyribonucleotides or polyribonucleotides are involved, the secondary structure plays an important role in their antigenic specificity.

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Transfer RNA complexed or conjugated with a protein carrier induces anti-RNA antibodies (PLESCIA et al., 1965b; BONAVIDA et al., 1970). In contrast, if the ribonucleic acid is injected without a carrier the antibodies elicited react with oligonucleotides (HERNANDEZ et al., 1968) but not with tRNA. Mycophage RNA emulsified in complete Freund's adjuvant induces antibodies to RNA in NZB/NZW mice (TALAL et al., 1971).

In order to give DNA immunizing properties PLESCIA et al. (1964, 1965) complexed the material to MBSA. A certain number of polynucleotides such as denatured DNA, poly dAT, and tRNA associated with MBSA have been used as immunogens by these authors. This method of preparation has been used for other polynucleotides, particularly for single-stranded synthetic polynucleotides and the various double- and triple-helical complexes formed from such polyribonucleotides. It appears that secondary structure of these complexes is generally maintained on adsorption onto MBSA, although some of their characteristics could well be modified in this electrostatic interaction.

In order to elucidate the role of conformation of the immunogen, animals were immunized with complexes of polyribonucleotides (double- or triple-stranded) associated with MBSA. The antibodies obtained reacted specifically with double- or triple-stranded complexes and it was concluded that the specificity was determined by the macromolecular conformation of the immunogen (NAHON et al., 1967; LACOUR et al., 1968; MICHELSON et al., 1971). Immunization of rabbits with DNA-MBSA or with double-stranded polynucleotide complexes adsorbed to MBSA elicits antibodies belonging to the macroglobulin class (STOLLAR and SANDBERG, 1966; NAHON-MERLIN et al., 1973).

Antibodies reacting preferentially with certain kinds of natural RNA, or with certain polydeoxyribonucleotide complexes, and with hybrid complexes, have been obtained by immunization with double-stranded polynucleotide complexes (NAHON-MERLIN et al., 1971; STOLLAR, 1970).

The purpose of this chapter is to present the results obtained with different double-stranded helical polynucleotide models, and to describe the progress which has been realized in the immunological recognition of the structure of polynucleotides.

2. Definition and Immunogenicity of Poly I. Poly C

In aqueous solution the two homopolynucleotides poly I and poly C readily associate to give a double helical complex poly I · poly C (DAVIES and RICH, 1958). The stoichiometry of this complex has been established by a variety of techniques and at pH 7 only the double-stranded complex is obtained. No triple-stranded structure has been demonstrated under these conditions. The stability of the complex is a function of salt concentration and in 0.15 M Na^+, pH 7.0, the temperature of dissociation of the two strands is about 60°. The complex is a right-handed helix and appears to have a geometry similar to that of the A form of RNA.

When this synthetic polyribonucleotide complex, poly I · poly C adsorbed onto methylated serum albumin is used as immunogen, antibodies reacting

with the complex can be induced in three varieties of rodent: rabbits (NAHON et al., 1967; SCHUR and MONROE, 1969; STOLLAR, 1970; KOFFLER et al., 1971), mice and hamsters (LACOUR et al., 1971).

Even without carrier protein or adjuvant, poly I · poly C appears to be in itself immunogenic in mice. Thus, STEINBERG et al. (1969, 1971) having observed the appearance of antibodies reacting with poly I · poly C in New Zealand mice after repeated injections of the complex, studied the effect of 8 to 12 injections (each of 70 to 150 μg) of the complex into NZB/NZW F1 (B/W) mice, and also ALN mice, strains that spontaneously produce antibodies that have the capacity to react specifically with radioactive poly I · poly C. As controls three other strains of mice, C3H/He, C57 BL/6 and BALB/c, were used. Although the presence of spontaneous anti-nucleic acid antibodies can be explained by immunomodifying factors in B/W mice that spontaneously develop an autoimmune disease similar to systemic lupus erythematosus (SLE) in man, the results obtained with immunologically apparently normal ALN mice remain unexplained.

A very low level of antibodies has been observed in B/W mice after 8 injections, and after 12 injections the average level of antibodies reacting with ^{14}C- poly I · poly C was 3.9 μg/ml in male mice and 15.9 μg/ml in the females, that is, higher than in non-treated mice of the same strain and of the same age. In contrast, the level of antibodies reacting with poly I · poly C is the same in treated or non-treated ALN mice and it is zero in treated mice of three other strains used as controls. Thus only B/W mice respond to repeated injections of poly I · poly C by production of antibodies reacting with the homologous antigen. Although the animals were immunized at the age of one month when anti poly I · poly C antibodies could not be detected and the mice showed no clinical signs of autoimmune disease, it is nevertheless possible that in this case an acceleration of a spontaneous process is involved. Poly I · poly C alone did not induce the production of antibodies either in C3H/He, C57Bl/6, BALB/c (STEINBERG et al., 1971) or in RAP mice (LACOUR et al., 1971).

The results are different when poly I · poly C is injected in the presence of complete Freund's adjuvant. For example, a single injection of 150 μg of the complex in emulsion with an equal quantity of adjuvant induces an average of 116 μg/ml of anti poly I · poly C antibodies in male and some 181 μg/ml in female B/W mice, aged one month. The quantity of antibodies is thus not negligible, and is higher in the female than in the male. The hypothesis of a genetic regulation of the immunological response to synthetic polynucleotides has been suggested by STEINBERG et al. (1971). This hypothesis has also been evoked by LACOUR et al. (1971), to explain the difference in specificity of antibodies obtained in rabbits, mice and hamsters with the same preparation of immunogen, poly I · poly C-methylated serum albumin.

Although all the results agree with respect to cross-reactions between poly I · poly C antibodies and synthetic poly A · poly U, reactions that are nearly always observed with immune sera of rabbits and mice (NAHON et al.,

1967; SCHUR and MONROE, 1969; STOLLAR, 1970; KOFFLER et al., 1971; STEINBERG et al., 1969; LACOUR et al., 1971) and somewhat less frequently with immune sera of hamsters, the specificity of the antibodies with respect to nucleic acids varies widely (LACOUR et al., 1971). This difference in specificity can be explained if one considers the possibility that the antigen may undergo various modifications *in vivo* and in particular enzymatic degradation, which varies from one species to another and even among different strains of a given species. Since the specificity of the antibodies varies according to the animal used, it will be more convenient in the following section, to discuss successively the characteristics of anti-poly I · poly C antibodies of different species.

3. Specificity of Anti-Poly I. Poly C Antibodies Induced in the Rabbit

Prior to discussing the specificity of anti-poly I · poly C antibodies it will be useful to refer briefly to results obtained when the single-stranded polynucleotides, poly I and poly C, are used as immunogens. Rabbits immunized with these homoribopolynucleotides bound to MBSA produce antibodies directed essentially against the homologous polyribonucleotides, together with a very small amount of anti-MBSA antibodies. The specificity of the anti-polynucleotide antibodies is associated with the purine or pyrimidine base, and the anti-poly I antibodies react particularly with poly I while the anti-poly C antibodies react only with poly C. This specificity appears to be very strict and cross-reactions with heterologous polyribonucleotides are exceptional (SEAMAN et al., 1965). Anti-poly I and anti-poly C antibodies also react with denatured DNA. The antibodies to single-stranded polynucleotides are incapable of recognizing the homologous antigen when it is involved in a hydrogen-bonded double-helical structure, for example in poly I · poly C (NAHON et al., 1967a, b). Similarly INOUYE et al. (1971) have shown that anti-inosine antibodies which react with poly I do not react with poly I · poly C except after destruction of the double-strand by selective cleavage with pancreatic ribonuclease.

Poly I · poly C complexed to MBSA is a good immunogen. It induces (in all the rabbits immunized) antibodies reacting with poly I · poly C and with at least one of the components of the complex. These antibodies also recognize other double-helical polyribonucleotide complexes (NAHON et al., 1967a, b; SCHUR and MONROE, 1969; KOFFLER et al., 1971) and also polydeoxyribonucleotide complexes (MICHELSON et al., 1971) as well as hybrid complexes between a polyribonucleotide strand and a complementary polydeoxyribonucleotide (STOLLAR, 1970). They also react with natural RNA. In such a system the molecular conformation and structure of the polyribosephosphate chain, as well as the presence of ribose, of certain bases, or of certain functional groups on these may play a role as antigenic determinant. It is thus possible to envisage the presence of antibodies with different specificities in these immune sera.

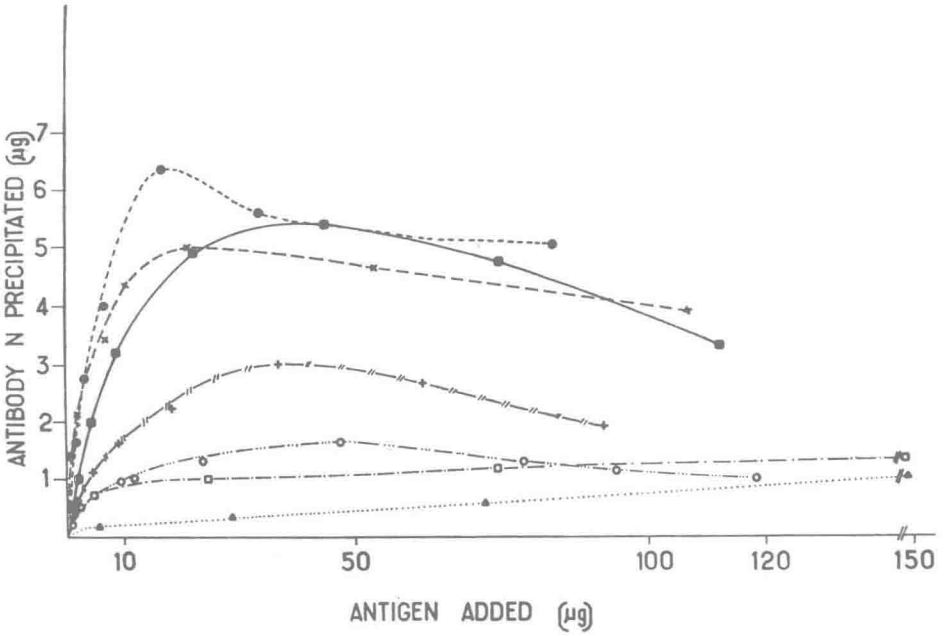

Fig. 1. Precipitin reaction of anti-poly I · poly C serum × 929 (0.5 ml of a 1:10 dilution) and various antigens. ●–––– ● poly I · poly C; x––––x poly A · poly U; □ –·–·– □ poly rG · poly rC; +–//–//–//–+ poly dG · poly dC; o–·····–·····–o poly I; ▲.... ▲ poly G; ■——■ total RNA from mouse ascites cells. Quantitative precipitin analyses were performed as described by KABAT and MAYER (1961) using the Folin-Ciocalteu method for analysis of specific precipitates

In order to define the specificity of the antibodies and to attempt to identify the antigenic determinants (or groups of antigenic determinants) NAHON-MERLIN et al. (1973a) studied the cross-reactions of anti-poly I · poly C antibodies with different polynucleotides and polynucleotide complexes by direct precipitation and by specific absorption of antibodies. The rabbits respond to immunization with poly I · poly C — MBSA, with a production of antibodies which varies according to the rabbit; the sera contain from 700 to 1000 µg/ml of antibodies.

Quantitative analysis of the reactions with other polynucleotides has demonstrated significant differences among three double-helical complexes (Fig. 1). Thus the complex poly rA · poly rU precipitates 99.9 µg/ml of antibodies (expressed as N) and shows a much greater reactivity compared with that of poly rG · poly rC which only precipitates 26.4 µg/ml of antibodies. In addition seven times more poly rG · poly rC than poly rA · poly rU is necessary to reach equivalence. This low activity of poly rG · poly rC could explain the negative results obtained with less sensitive techniques such as immunodiffusion in agar used in previous studies (NAHON et al., 1967; SCHUR and MONROE, 1969). It is interesting to note the best reactivity of the corresponding polydeoxyribonucleotide complex, poly dG · poly dC, which precipitates

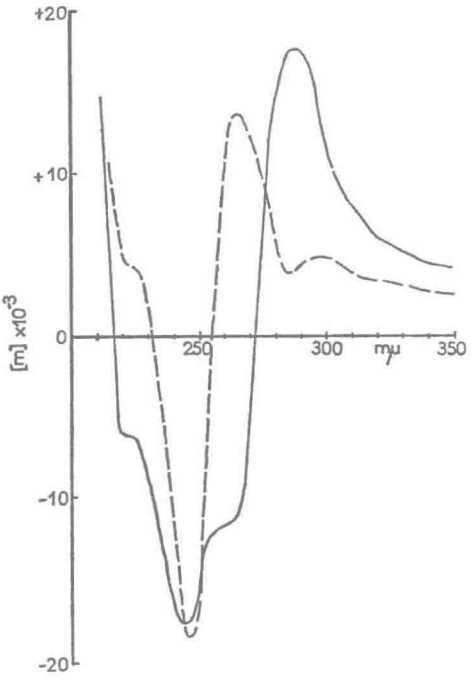

Fig. 2. Optical rotatory dispersion curves of poly G · poly C (———) and of poly dG · poly dC (– – – –) in 0.01 M NaCl, 0.005 M sodium cacodylate pH 7.0 at 20°

60 μg/ml of antibodies. There is thus an immunochemical difference on one hand between three polyribonucleotide complexes poly I · poly C, poly A · poly U and poly G · poly C and, on the other hand, between two complexes containing the same bases, guanine and cytosine, one of which is ribo- and the other polydeoxyribonucleotide. The fact that poly dG · poly dC precipitates more anti-poly rI · poly rC antibodies than does poly rG · poly rC is evidence in favor of the hypothesis that the specificity of the antibodies depends to a large extent on the conformation of the double-helix rather than on the presence of a given base or the nature of the sugar. Such immunochemical differences probably reflect differences of structure shown by circular dichroism and optical rotatory dispersion (Fig. 2 and 3) (MICHELSON et al., 1971).

Analysis of the specific absorption of anti-poly I · poly C antibodies by double-stranded polynucleotide complexes reinforces both the idea of specificity of these antibodies for double-stranded structures and also the immunochemical differences between the three double-helical complexes. Thus poly rA · poly rU, poly dG · poly dC and poly rG · poly rC absorb, respectively, 81.5, 73.3 and 57.7 % of the antibodies in the homologous system. In order to inhibit 50 % of the reaction with the homologous antigen, 5.7 μg of poly A · poly U, 12.5 μg poly dG · poly dC, and 85 μg of poly rG · poly rC

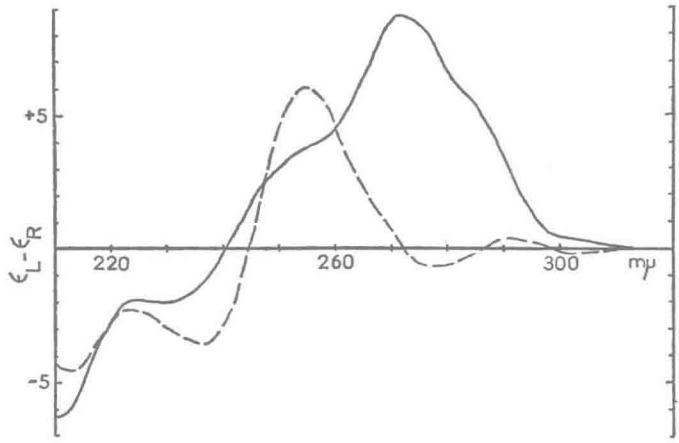

Fig. 3. Circular dichroic spectra of poly G · poly C (———) and of poly dG · poly dC (– – – –) in 0.01 M NaCl, 0.005 M sodium cacodylate pH 7.0 at 20°

Table 1. Quantitative analysis of the reaction between absorbed anti-poly I · poly C serum 929 and poly I · poly C

Antiserum absorbed with:	Amount of polynucleotide or polynucleotide complex required to absorb 50% antibody (μg)	Percent absorption at the maximal concentration of polynucleotide or polynucleotide complex used
Poly A · poly U	5.7	81.5
Poly dG · poly dC	12.5	73.3
Poly rG · poly rC	85	57.7
Poly C		46.9
Poly A		39.7
Poly G		36.4
Poly I		34.1
Poly U		22.0

are required (Table 1). In contrast none of the homopolynucleotides, poly A, poly U, poly C, poly I, or poly G, inhibit 50% of the reaction with poly I · poly C even if 10 to 40 times greater quantities of the inhibitor are used. Among polynucleotides not involved in specific complementary hydrogen bonds, poly U, poly C, poly A, poly I and poly G, only the last two are precipitated by immune sera to poly I · poly C. It may be noted that both poly I and poly G form structured helices, though in contrast with poly I · poly C, in which the strands are antiparallel, in poly I the strands are parallel and this is probably also true of poly G (see MICHELSON et al., 1967).

The antisera thus precipitate a polynucleotide which is one of the components of the immunogen and also a heterologous polynucleotide. The fact that anti-poly I · poly C precipitates poly I in single-stranded form suggests the possibility that the sera contain, in addition to antibodies specific for

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