Immune RNA in Neoplasia

Edited by

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Division of Cancer Research Resources and Centers
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Preface

The idea that ribonucleic acid (RNA) from an immunized host might incite a specific immune response in another host has been with us for some time. Over the last decade an active attempt has been made in several laboratories to demonstrate that this concept applies in neoplasia and that it holds promise of being a reliable immunotherapeutic approach. In order to accomplish this, however, a concerted effort needs to be made to understand exactly what immune RNA is–how to isolate, purify, and characterize it–and how and under what circumstances it can function to bring about a therapeutic response.

After listening to the presentations concerned with immune RNA at a recent national meeting, it became clear that there was considerable confusion regarding methodology and terminology to the extent that it was difficult to compare –and sometimes difficult to interpret–the results. Thus the idea arose that it might be profitable to hold a workshop in immune RNA to address these problems. After discussions with various people, the possible value of a dialogue between the biologists working with immune RNA and molecular biologists concerned with the fine points of characterization of RNA became apparent. With this in mind, the conference evolved under the enthusiastic co-chairmanship of Drs. Yosef H. Pilch and Robert J. Crouch. It was held at the Marine Biological Laboratory, Woods Hole, Massachusetts, October 8-11, 1975. This volume includes the papers presented and session chairmen's summaries of discussions, as well as two overviews.

For the success of the conference and this volume, we are indebted to a number of people: to Robert Crouch and his colleagues for their altruistic gift of time and energy to help solve the problems of scientists in a different area; to Stewart Sell for his unflagging objectivity; to Frances Cohen for excellent editorial assistance; to Barbara Huffman and other Division secretaries for their aid and support; and especially to Dennis Fink for his understanding and help throughout this endeavor.

AN INTRODUCTION TO IMMUNE RNA[1]

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What is Immune RNA?*

As is the case with many biological processes, the concept of "immune" RNA is rather straightforward. Cells incubated in the presence of immune RNA can be converted to the production of antibodies of the specificity of the cell from which the immune RNA is isolated. One of the earliest examples of this phenomenon was a demonstration by Fishman (1) of the transfer of immunological specificity employing T2 bacteriophage as antigen. Macrophages were incubated with bacteriophage T2, a lysate was prepared and filtered, and the filtrate was added to a culture of lymphnode cells. Antibody directed against T2 was generated and a search for the source of this magic substance began. Soon at least a partial explanation appeared. Fishman and Adler (2) demonstrated that if RNA is isolated from macrophages incubated with T2, this RNA can be used to convert the recipient cell to production of antibodies which inhibit T2 plaque formation. RNase destroyed the converting activity but not DNase.

Is immune RNA simply the carry over of antigen?

One of the most plausible and earliest explanations for the phenomenon of immune RNA is that an antigen (such as T2) is still present in the RNA extract and it is the antigen which elicits the formation of antibody in the lymphnode cells. The early reports of "immune RNA" seemed to exclude this possibility since the T2 antigen should be readily detectable and, moreover, T2 added to the lymphnodes did not result in detectable antibody. These results were challenged on two fronts: (a) RNA might act as an adjuvant (i.e., very low levels of T2 would be sufficient to stimulate T2 antibody in the presence of RNA and (b) T2 bacteriophage might be altered in such a way

[1] This summary of the status of immune RNA prior to the meeting on Immune RNA in Neoplasia at Wood's Hole, Massachusetts was presented with the intent that the reader would know some of the ideas and work in this area. A complete bibliography is not presented and references are presented which may omit some of the same work published by other workers.

that special techniques would be required to detect the modified antigen or (c) both of the possibilities might occur (e.g., modified bacteriophage associated with RNA). RNA-antigen complexes have indeed been reported in amounts which seemed to account for all of the immunological activity of immune RNA preparations (3). Subsequent studies (4) employing a similar procedure revealed a fraction of immune RNA that appeared to be free of any antigen yet the properties described for immune RNA remained intact. One of the most sensitive tests to demonstrate the absence of antigen in immune RNA utilized an antigen (mono-(p-azobenzene arsonate)-N-chloracetyl-L-tyrosine) [ARS-NAT] containing arsenic (5). By atomic absorption spectra, it was possible to say that immune RNA contained less than 0.0000065% by weight of ARS-NAT, yet was able to confer the ability to make antibody to ARS-NAT when incubated with appropriate cells.

Certainly, such a low level of contamination is an indication that only RNA is involved but, if the following calculation is made, even this low level does not exclude the involvement of antigen. Since most of the RNA isolated from these cells is ribosomal or t-RNA, all but five percent of the RNA should be excluded from these calculations. Starting with 1 mg of total RNA we then exclude ribosomal and t-RNA giving us 50 μg of RNA. Estimates of the size of immune RNA, as determined by sucrose gradient sedimentation are on the order of 14-18S (6) or about 2,000 nucleotides. If we ascribe all 50 μg of RNA as being immune RNA, the following calculation can be made:

$$\frac{50 \times 10^{-6} \text{ g RNA}}{2 \times 10^{3} \text{ nucleotides } .320 \text{ g/mole nucleotide}} = 7.81 \times 10^{-11} \text{ moles immune RNA}$$

$$6.5 \times 10^{-8} \text{ g ARS-NAT/g RNA}$$

or 6.5×10^{-8} mg ARS-NAT/mg RNA

$$\frac{6.5 \times 10^{-11} \text{ g ARS-NAT}}{486 \text{ g/mole ARS-NAT}} = 1.11 \times 10^{-13} \text{ moles ARS-NAT}$$

$$\text{giving } \frac{11.1 \times 10^{-14} \text{ moles ARS-NAT}}{7.81 \times 10^{-11} \text{ moles immune RNA}} = 1.04 \times 10^{-3} \frac{\text{moles ARS-NAT}}{\text{moles immune RNA}}$$

or 1 molecule of ARS-NAT per 1000 molecules of immune RNA.

There are, of course, limits to this calculation. First, we have assumed that all of the non-ribosomal and t-RNA is immune RNA which can only be an overestimate of the ratio of moles ARS-NAT/moles immune RNA. Second, the ARS-NAT value used is the maximal amount possible and would give an underestimate of moles ARS-NAT/moles immune RNA. It seems unlikely that all 50 μg of RNA is immune RNA, at least immune RNA specific for

ARS-NAT; and that one molecule of ARS-NAT per 1000 molecules of immune RNA might represent one molecule of ARS-NAT per one molecule of immune RNA specific for ARS-NAT. The purpose of this calculation is to demonstrate the difficulty of ever resolving the question of antigen contamination by this approach.

Genetic evidence supporting the concept of immune RNA

Experiments that give the strongest support for the concept of immune RNA are based on genetic differences among immunoglobulins of different animals. Slight changes in the amino acid sequence of the immunoglobulin molecule from one rabbit (A) to a second (genetically distinct) rabbit (B) have been demonstrated. Furthermore, it is possible to prepare antibodies to these different immunoglobulins (which are now being utilized as antigens) and make antisera specific for either rabbit A or rabbit B type immunoglobulins. Differences of this sort are called allotypic and show normal genetic inheritance on breeding. Allotypic differences permit the following class of experiments to be performed: immune RNA from a rabbit of allotype A can be incubated with cells from a rabbit of allotype B and the immunoglobulins produced in these cells can be challenged with antisera against type A or type B immunoglobulins. If immune RNA from type A cells is carrying the antigen into the cells of type B, the immunoglobulin produced in type B cells should be of B allotype and not A allotype. On the other hand, if immune RNA is carrying information for the production of immunoglobulins, it might be expected that immunoglobulins produced in cells (which are of B allotype) would have allotypic characteristics of the A type. The results of such experiments (7,8) clearly demonstrate the conversion of cells of B allotype by immune RNA of A allotype to the production of immunoglobulins of A allotype. A straightforward explanation of these results based on the carry over of antigen in immune RNA is difficult to conceive.

It follows from the experiments just described that immune RNA from type A cells carries information concerning the structural features of type A immunoglobulins, including both the allotypic determinants and the antigenic specificity. Several lines of evidence have suggested that mRNA for immunoglobulins is the vehicle for this information transfer. First, the "converting" activity is sensitive to RNase but not DNase or pronase. Second, drugs which block protein synthesis, but not those that block RNA synthesis, are able to prohibit conversion of a cell to produce immunoglobulins of the immune RNA type. Third, this material is isolated by techniques normally employed in the isolation of RNA. Fourth, immune RNA sediments in a sucrose gradient in the region of mRNA for immunoglobulins (6). Fifth, immune RNA binds to oligo(dT)-cellulose columns (6), a technique frequently used to isolate many types of mRNA from eukaryotic systems. Finally, some evidence is

being developed which indicates that immune RNA can be translated in an *in vitro* protein synthesizing system to produce active immunoglobulins (9).

Detection of changes induced by immune RNA

This brief outline of some of the properties of immune RNA is based on experiments utilizing a variety of animals and assays for the detection of the changes induced by immune RNA. Unlike most of the assays that molecular biologists use, in which there is a very simple and direct assay of the product (e.g., incorporation of ATP into RNA by RNA polymerase), most immunological assays use properties of the immunoglobulins (e.g., antibodies against T2 bacteriophage are detected by the inhibition of phage formation of T2 - not an assay that is for the T2 antibody complex).

Probably the greatest difficulty that a molecular biologist encounters in understanding experiments concerning immune RNA lies in the techniques of the system and the estimation of the validity of such techniques. Papers presented in this volume are based on experiments in which techniques of immunoglobulin detection range from inhibition of T2 phage plaque formation to the Jerne plaque technique to a rosette assay and to the production of migration inhibitory factor (MIF). A brief description of these assays follows and reflects a "text book" interpretation to generate some basic understanding of immunological properties exploited in these assays. Inhibition of T2 plaque formation represents the more classical demonstration of antibody production in which the antigen and antibody are permitted to interact and the formation of antibody is measured by a decrease in the titer of the phage. The rosette procedure is described in an accompanying paper (10). The Jerne plaque assay involves plating antibody producing cells – in this case converted to specific antibody production by immune RNA – on a lawn of sheep red blood cells (SRBC). If the RNA is from cells or animals immunized with SRBC, addition of complement to the antigen-antibody complex results in hemolysis of the SRBC, and a cell producing antibody to SRBC is observed as a clear plaque in the lawn of SRBC. Assays employing MIF rely on the observation that the motility of macrophages is inhibited in the presence of antigen for which there is a corresponding type of macrophage (11). Migration of all macrophages is presumably inhibited if any "cells" are specifically stimulated to make MIF by the antigen. As an example, if sensitive cells are stimulated by ARS-NAT, addition of this antigen to macrophage-containing cell suspensions in a capillary tube will inhibit migration of the macrophages from the capillary tube. Each system relies on slightly different immunological properties to detect specific antibody production and has different levels of sensitivity. Theoretically, the T2 system could detect one molecule of antibody, and the Jerne plaque assay or the rosette assay may detect single cells producing antibodies. The actual level of resolution in these systems is far from theoretical and, in many cases, quantitation is relative.

Immune RNA and myeloma proteins

In the past few years, immunologists have brought the study of myeloma proteins to an extremely useful state. A series of recent papers utilizing myelomas has tended to give strong support to the concept of immune RNA as well as to provide evidence that immune RNA is identical to mRNA for immunoglobulins. Since myelomas produce a single species of immunoglobulin, immune RNA obtained from these tumors should transfer very specific information to the recipient cells. Certainly, the mRNA for immunoglobulins in these cells direct the *in vitro* synthesis of the myeloma protein. Transfer of this mRNA to a recipient cell should convert that cell to the production of immunoglobulins of the myeloma type. At this point another bit of terminology must be considered. Alterations in the immunoglobulin molecule which allow the antibody to interact with an antigen (or, more precisely, a portion thereof) are thought to occur such that the portion of the immunoglobulin interacting with the antigen is constant from one molecule to another, irrespective of the allotypic characteristics of the immunoglobulin. These determinants are said to specify the idiotype of an immunoglobulin molecule. In contrast to allotype, in which all immunoglobulins from an individual rabbit of genotype A possess the allotypic determinants A but many different antigenic responses, all immunoglobulins of idiotype I should contain a common region which is dependent on its antigenic specificity. Myeloma proteins represent a situation in which a cell is producing proteins of one idiotype. If these myeloma proteins are used as antigens to immunize syngeneic hosts, antibodies are produced which interact specifically with the region containing the idiotypic determinants. Such antibodies are said to be anti-idiotypic. Antibodies of this type are extremely useful in detecting the production of myeloma proteins and make the myeloma system amenable to manipulation of immune RNA.

RNA extracted from myeloma tissue can cause lymphocytes from animals which are free of myelomas and are not producing myeloma protein to begin the production of immunoglobulin molecules with idiotypic characteristics of the myeloma protein from which the immune RNA is extracted (12). Again it has been shown that such transfer of information can be inhibited by pretreatment of the immune RNA with RNase but not DNase or pronase. Establishment of the conversion is inhibited by cycloheximide and puromycin but not actinomycin D.

It is known that lymphocytes from animals bearing myelomas have surface immunoglobulins of the myeloma type and such animals have a greatly reduced ability to respond to antigens (i.e., they are immunosuppressed). RNA from myelomas is able to confer on animals the same state of immunosuppression observed in the diseased animal and, in addition (13), attempts to convert cells which are immune suppressed with immune RNA have been successful (14). These results suggest that immunosuppression occurs as a result of the

disease and, in some manner, the lymphocytes of the diseased animal are exposed to a substance similar (or identical) to immune RNA. Particles have been observed in the plasma of animals with plasmacytomas which can convert normal lymphocytes to lymphocytes whose surface immunoglobulins are the plasmacytoma type (15). Also plasma has yielded immune RNA with the ability to convert cells to lymphocytes bearing plasmacytoma proteins (17).

Stable conversion by immune RNA

Is it possible to use immune RNA to convert cells to a new, genetically distinct immunoglobulin type? Immunosuppression seems to indicate that plasmacytomas have altered the normal immune system in a stable manner. But will the same be true if nontumor immune RNA is utilized? Phenotypic changes due to immune RNA do not depend on a cellular system sensitive to actinomycin D but protein synthesis inhibitors prevent conversion. A stable or long term change should, in some way, depend on a process that amplifies the immune RNA. Bhoopalam *et al.* (16) have performed a serial passage of immune RNA to test for increased amounts of immune RNA. RNA was extracted from a plasmacytoma and injected into a disease-free mouse. After one hour the spleen was removed from the mouse, one-half of the spleen was put into culture for 7 days or extracted immediately to yield immune RNA. *In vitro* conversion was demonstrated with the RNA isolated from the spleen immediately after removal of the spleen; but more conversion was seen with the RNA isolated from the cells that had been cultured for seven days. Also RNA prepared in a similar manner was passed sequentially through 5 animals each time injecting immune RNA in the mouse then extracting the RNA from the spleen, injecting that RNA into a second mouse, and so on. Conversion activity was lost after two or three passages when the "immediate" extract RNA was used but even after 5 passages with "7 day" immune RNA cell converting activity remained. These results were taken to indicate RNA replication in the recipient cells. Previous experiments (17) demonstrated a similar transfer but there was no genetic evidence to exclude antigen contamination.

Comparison with other systems

Attempting to understand the mechanism involved in what can almost be described as cellular transformation (here phenotypic changes and viral production will be included as examples of transformation) with RNA requires some new assumptions not previously invoked to explain other transformation. If immune RNA simply enters the cell and becomes an active mRNA without any amplification of the immune RNA either via an RNA or DNA intermediate, then we have to wonder why the cell takes up this RNA, why the cell is converted to production of proteins with this immune RNA, and why once converted the cell seems to be resistant to further change (i.e., becomes immune suppressed).

There are many examples of bacterial cells transformed with DNA (18) or transfected with bacteriophage DNA (19) or RNA (20). Polio RNA is infectious (21), DNA of Adenovirus 2 can transform cells (22), and there are other demonstrations of DNA or RNA transformation. All of these examples are easily understood. Transformation of bacteria by DNA simply involves the host mechanism of recombination. Most examples of viral nucleic acid can be explained in terms of entry of the nucleic acid into the cell. In most instances nucleic acid is injected or carried into the cell via the virus particle. Once inside the cell, the free nucleic acid is able to direct the synthesis of new viruses. A good example of this is shown by a comparison of poliovirus and vesicular stomatitis virus (VSV). Poliovirus contains the strand of RNA which can be translated into poliovirus proteins, whereas VSV contains the strand complementary to the translatable strand, along with an RNA dependent RNA polymerase to generate the translatable strand. RNA from poliovirus is infectious while VSV RNA is uninfectious.

There are enzymes capable of amplifying immune RNA, either a RNA dependent DNA polymerase or a RNA dependent RNA polymerase (23-26). When RNA is isolated from nonimmune animals or from tissues such as liver, cellular conversion to new types of immunoglobulins does not occur. Are these RNAs taken up and treated in the same way as immune RNA? Could globin mRNA be translated and replicated in this system?

There is one other phenomenon in which either DNA or RNA is used to transform a cell. In no case is viral nucleic acid as effective as the normal mechanism of infection. Lambda DNA transfects *E. coli* with an efficiency of one in 10^5 and polio RNA is about a thousand fold less efficient than poliovirus. Very few, if any, biochemical techniques are available which can tell us much about those nucleic acid molecules that are infectious. Will the efficiency of utilization of immune RNA be any different? Clearly, a technique which permits the survival of most of the immune RNA would, from a biochemical approach, be extremely useful. Micro injection techniques are available and should be a tool of great importance for solving some of the problems of immune RNA biochemistry.

REFERENCES

1. Fishman, M. (1961). Antibody formation *in vitro*. *J. Exp. Med. 114*:837-856.

2. Fishman, M. and F. L. Adler (1963). Antibody formation initiated *in vitro*. II. Antibody synthesis in x-irradiated recipients of diffusion chambers containing nucleic acid derived from macrophages incubated with antigen. *J. Exp. Med. 117*:595-602.

3. Gottlieb, A. A. and R. H. Schwartz (1972). Review – antigen-RNA interactions. *Cell. Immunol. 5*:341-362.

4. Fishman, M. (1973). The role of macrophage RNA in the immune response. *The Role of RNA in Reproduction and Development.* Niu and Segal, Eds. North-Holland Publishing Co.

5. Schlager, S. I., S. Dray and R. E. Paque (1974). Atomic spectroscopic evidence for the absence of a low-molecular weight (486) antigen in RNA extracts shown to transfer delayed-type hypersensitivity *in vitro*. *Cell. Immunol. 14*:104-122.

6. Giacomoni, D., V. Yakulis, S. R. Wang, A. Cooke, S. Dray and P. Heller (1974). *In vitro* conversion of normal mouse lymphocytes by plasmacytoma RNA to express idiotypic specificities on their surface characteristic of the plasmacytoma immunoglobulin. *Cell. Immunol. 11*:389-400.

7. Adler, F. L., M. Fishman and S. Dray (1966). Antibody formation initiated *in vitro*. III. Antibody formation and allotypic specificity directed by ribonucleic acid from peritoneal exudate cells. *J. Immunol. 97*:554-563.

8. Bell, C. and S. Dray (1969). Conversion of non-immune spleen cells by ribonucleic acid of lymphoid cells from an immunized rabbit to produce γM antibody of foreign light chain allotype. *J. Immunol. 103*:1196-1211.

9. Bilello, P. A., G. Koch and M. Fishman (1975). mRNA activity of immunogenic RNA. *Fed. Proc. 34*:1030.

10. Heller, P., N. Bhoopalam, Y. Chen and V. Yakulis (1976). The relationship of myeloma "RNA" to the immune response. *Immune RNA in Neoplasia.* Mary A. Fink, Ed. Academic Press, New York.

11. Weiser, R. L., Q. N. Myrvik and N. N. Pearsall (1969). *Fundamentals of Immunology*, pp. 196-197. Lea and Febiger, Philadelphia.

12. Bhoopalam, N., V. Yakulis, N. Vostea and P. Heller (1972). Surface immunoglobulin of circulating lymphocytes in mouse plasmacytoma. II. The influence of plasmacytoma RNA on surface immunoglobulins of lymphocytes. *Blood 39*:465-471.

13. Yakulis, V., V. Cabana, D. Giacomoni and P. Heller (1973). Surface immunoglobulins of circulating lymphocytes in mouse plasmacytoma. III. The effect of plasmacytoma RNA on the immune response. *Immunol. Comm. 2*:129-139.

14. Adler, F. L. and J. Fishman (1975). *In vitro* studies on information transfer in cells from allotype-suppressed rabbits. *J. Immunol. 115*:129-134.

15. Katzmann, J., D. Giacomoni, V. Yakulis and P. Heller (1975). Characterization of two plasmacytoma fractions and their RNA capable of changing lymphocyte surface immunoglobulins (cell conversion). *Cell. Immunol. 18*:98-109.

16. Bhoopalam, N., V. Yakulis, D. Giacomoni and P. Heller. Surface immunoglobulins of lymphocytes in mouse plasmacytoma. IV. Evidence for the persistence of the effect of plasmacytoma-RNA on the surface immunoglobulins of normal lymphocytes *in vivo* and *in vitro*. *Clin. Exp. Immunol.*, in press.

17. Saito, K., S. Kurashige and S. Mitsuhoshi (1969). Serial transfer of immunity through immune RNA. *Jap. J. Microbiol. 13*:122-124.

18. Avery, O. T., C. M. MacLeod and M. McCarty (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types. *J. Exp. Med. 79*:137-158.

19. Kaiser, A. D. and R. Wu (1968). Structure and function of DNA cohesive ends. *Cold Spring Harbor Symp. 23*:329-734.

20. Pace, N. R. and S. Spiegelman (1966). The synthesis of infectious RNA with a replicase purified according to its size and density. *Proc. Nat. Acad. Sci., USA 55*:1608-1615.

21. Koch, G. (1973). Stability of polycation-induced cell competence for infection by viral RNA. *Virology 45*:841-843.

22. Graham, F. L. and A. J. von der Eb (1973). A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology 52*:456-467.

23. Jacherts, D., U. Opitz and H-G. Opitz (1972). Gene amplification in cell-free synthesis. *Z. Immun.-Forsch., Bd 144*:s. 260-272.

24. Kurashige, S. and S. Mitsuhashi (1973). The possible presence of ribonucleic acid-dependent deoxyribonucleic acid polymerase in the immune response. *Japan. J. Microbiol. 17*:105-109.

25. Saito, K. and J. Mitsuhashi (1973). Ribonucleic acid-dependent ribonucleic acid replicase in the immune response. *Japan J. Microbiol. 17*:117-121.

26. Downey, K. M., J. J. Byrnes, B. S. Jurmark and A. G. So (1973). Reticulocyte RNA-dependent RNA polymerase. *Proc. Nat. Acad. Sci. USA 70*:3400-3404.

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