## Induction of Anti-*Treponema pallidum* Antibodies in Normal Rabbits by RNA-Immuno-Carrier Extracted from Serum of Syphilitic Rabbits

Antibody synthesis has been related to the production, by spleen and lymph-node cells of immunized animals, of RNA capable of inducing the formation of specific antibodies in normal cells[1-4].

MICHELAZZI et al.[5-10] found in the blood of immunized animals a RNA carrier of the antibody template which is able to induce the production of antibodies against the same antigens used for immunizing the animals source of immune RNA both in vivo and in vitro (RNA-immuno-carrier or RNA-I-C). In this investigation we tried to transfer anti-*Treponema pallidum* immunity from syphilitic rabbits to normal ones by RNA-I-C extracted from the serum of experimentally infected rabbits.

Syphilitic sera were prepared by injecting the rabbits intratesticularly with two 1 ml aliquots of a freshly prepared suspension containing approximately $1 \cdot 10^8$ viable *T. pallidum* (Nichols strain). Initial bleedings were taken before and after injection, periodically, to test the antibody titres. Between the 25th and 30th day, the syphilitic rabbits were sacrificed by bleeding and the blood collected; the RNA from the sera was immediately extracted by a slightly modified[10] phenol method described by KIRBY[11] and CHARGAFF[12]. An aliquot of RNA so prepared was treated with ribonuclease (0.5 $\mu$g/mg RNA at 37 °C for 30 min). The non-treated and ribonuclease treated RNA preparations were administered to different groups of normal rabbits by a single i.v. injection in the amount of 1.5 mg/kg body weight; a third group of animals, used as a control, was treated with the same volume of 0.14 *M* NaCl. Antibody titres of the pooled sera of all the animals of every group were tested 2 and 15 days after RNA injection. The RNA preparations were subjected before use to the same diagnostic tests used for the rabbit sera.

Serologic tests used to detect anti-*T. pallidum* antibodies were the usual diagnostic tests for the modern laboratory diagnosis of syphilis: VDRL slide flocculation test, Cardiolipin and Reiter protein complement fixation test (CCF, RPCF), *T. pallidum* immobilization test and fluorescent treponemal antibody test (TPI and FTA), all performed with the usual technique[13-15]. In addition, the *T. pallidum* agglutination test (TPA) was made according to the last technical findings[16]. The amount of antibody detectable with some of these tests was 0.011–0.065 $\mu$g antibody N in 0.05 ml serum[17] for the VDRL test and up to 0.014 $\mu$g antibody N for Wassermann reaction[18].

Serum of rabbits injected with viable *T. pallidum* show a progressively increasing titre of antibodies reaching the maximum on about the 30th day. At this time almost every diagnostic test reached the maximum: the behaviour of antibody titres during the period of immunization is summarized in Table I. The amount of RNA extractable from the serum of infected rabbits was, on the average, 5.45 mg/100 ml fresh serum, and its protein content was less than 0.07%.

Serum from rabbits used as recipients for RNA-I-C anti-*T. pallidum* was always non-reactive to every diagnostic test used, before RNA injection; but the serum from the same rabbits 2 days after injection of RNA-I-C from syphilitic rabbits had a clear reactivity, a bit lower for the CCF test but nearly maximum for all the other tests; 15 days after injection this reactivity almost disappeared, except for a weak reaction to VDRL and FTA tests.

Rabbits injected with RNA-I-C preincubated with ribonuclease were always non-reactive both after 2 and 15 days.

The RNA-I-C preparation was subjected before injection to all the same diagnostic tests as the serum to show antibody residues; but it was always negative (Table II).

Table I. Development of antibody response evaluated with several diagnostic tests by rabbits infected with *Treponema pallidum* (Nichols strain)

| Days after injection | VDRL | CCF | RPCF | TPI (% immobilization | FTA | TPA |
|---|---|---|---|---|---|---|
| 2 | – | – | – | 4% | – | – |
| 5 | – | – | ++ | 8% | +++ | ++++ |
| 10 | + | ++ | +++ | 8% | ++++ | ++++ |
| 15 | +++ | +++ | ++++ | 40% | ++++ | ++++ |
| 30 | ++++ | ++++ | ++++ | 70% | ++++ | ++++ |
| 60 | ++++ | ++++ | ++++ | 75% | ++++ | ++++ |

Table II. Serologic test results for laboratory diagnosis of syphilis from serum of syphilitic rabbits, normal rabbits, and normal rabbits injected with RNA-I-C extracted from serum of syphilitic rabbits

| Diagnostic tests | Syphilitic rabbits | Normal rabbits | Rabbits injected with RNA-I-C from serum of syphilitic rabbits | | Control RNA-I-C before injection |
|---|---|---|---|---|---|
| | | | 2 days after injection | 15 days after injection | |
| VDRL | ++++ | – | +++ | + | – |
| CCF | ++++ | – | + | – | – |
| RPCF | ++++ | – | +++ | – | – |
| TPI % immobilization | 70% | 4% | 65% | 16% | 4% |
| FTA | ++++ | – | +++ | + | – |
| TPA | ++++ | – | +++ | ± | – |


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The injection of viable *T. pallidum* (Nichols strain) intratesticularly in the rabbit, causes a specific orchitis and a strong antibody reaction against *T. pallidum* in toto and its components. Antibody titres increase until the 30th day after challenge, then remain almost unchanged.

RNA extracted from the serum of rabbits infected with *T. pallidum* is able, if administered i.v., to induce antibody response within 48 h. This is perfectly comparable, qualitatively, with that of directly infected rabbits 30 days after challenge, although it is lower in absolute value.

Therefore, there seems to be present in the blood of syphilitic rabbits a RNA carrier of the antibody template able to induce precocious antibody synthesis in the recipients. Otherwise in preliminary experiments the RNA extracted from non-syphilitic rabbits had no action and antitreponemal RNA loses its inducing power when treated with ribonuclease.

The antibody production cannot be considered as passive transport of preformed antibodies from the serum of the infected rabbits because: (1) the protein content of the RNA preparation used by us was very little; (2) RNA preparation, before use, was subjected to every diagnostic test with negative results; (3) besides, even if a minimal trace of antibody substance was present, it would be so diluted in the recipient animals that it would be unable to give an evident reactivity; (4) on the other hand, if traces able to give reactivity were present, these would appear at once and not after 48 h, as we always found both in this case and in our previous work on this subject[5].

Antibody production cannot be determined by the transport of antigenic substances from syphilitic rabbits to the recipient ones. In fact, even if traces of antigens were present in the RNA preparation they would be very little and unable to give such an evident and precocious response; and even if RNA conjugated antigens had a high antigenic capacity, the antibody response would be detectable only several days after challenge[19]. Also, it is well known that active immunization due to the presence of *T. pallidum* or its constituents occurs, usually, in the following way: first appear fluorescent antibodies, then antiprotein antibodies, the antilipidic antibodies and, lastly, the immobilizing antibodies (see Table I). In our case every type of antibody appeared simultaneously and likewise simultaneously disappeared, while it is known that in active immunization some antibodies (e.g. fluorescent and immobilizing) disappear later. So the hypothesis of an active immunization following the introduction of antigenic residues can be rejected.

*Zusammenfassung.* Es wird bestätigt, dass die i.v. Injektion von RNA aus dem Serum Syphilis-infizierter Kaninchen in gesunde Kaninchen die Bildung der Abwehrstoffe gegen *T. pallidum* verursacht.

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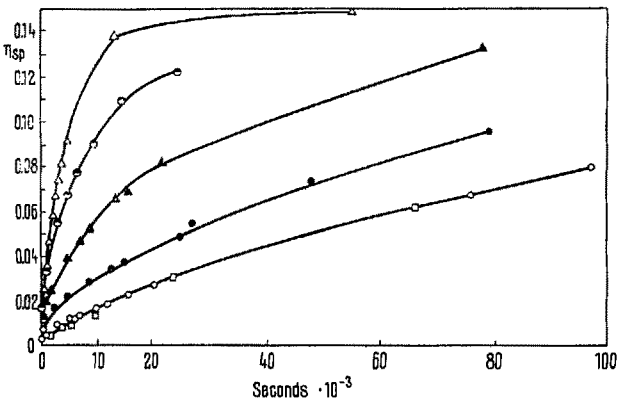
## The Low Temperature Reaction of Poly U and Poly A

It has been shown that, in the reaction of polyribouridylic acid (poly U) with polyriboadenylic acid (poly A) at room temperature to form a 2-stranded helical complex[1,2], a slow increase in the radius of gyration of the product, as measured by light scattering[3], persists for several hours, an effect that has been attributed to the annealing of imperfect bonding resulting in a molecule in which the maximum number of hydrogen-bonded base pairs have been formed. Since poly U undergoes a low temperature coil → helix transition[4], it was of interest to see the effect of varying helical content of poly U upon the reaction.

Accordingly, solutions of poly U and poly A (obtained from Miles Laboratories) in 1 *M* KCl, pH 7, were incubated at various temperatures and mixed in a viscometer in approximately a 1:1 ratio. Under these conditions only the 1:1 complex is formed at equilibrium. The viscosity was then read as a function of time. The results are shown in the Figure and clearly show that low temperature decidedly slows the annealing reaction. Parallel measurements of the absorbance ($A_{260\,nm}$) changes of identical but diluted reaction mixtures showed that almost all of the final absorbance value is reached within about a minute even at the lowest temperature studied.

A further effect of the helicity of poly U on the reaction between poly U and poly A is the monotonic decrease in the change in apparent hypochromism as the temperature




Time dependent changes in viscosity of mixtures of poly U + poly A as a function of temperature. Concentration (U + A) about 2 mg/ml. Solvent: 1 *M* KCl, 0.01 *M* lysine, pH 7.0. In this solvent the $T_m$ of poly U's coil-helix transition as measured by several techniques is 7.5 °C, in good agreement with the results of LIPSETT[4]. All curves eventually reach the same limiting value ($n_{sp}$ = 0.15) though at − 2 °C it takes nearly 5 days to do so. ○ = − 2 °C, □ = + 0.05 °C, ● = + 3 °C, ▲ = + 6 °C, ◓ = + 9 °C, △ = + 12 °C.

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