# Effect of Immune and Normal RNA on the Immunological Response Induced by Rat Red Blood Cells in Mice

In a previous paper[1] the authors studied the immunity against a rat sarcoma (Sarcoma E 100) induced by RNA from immune rats. It was postulated that the immune RNA could be instrumental in evoking a rapid response, thus probably transforming a first set immunological reaction into a second set one. It was, moreover, observed in some of the experiments that rats receiving normal RNA had larger tumours than control rats suggesting some type of tolerance. Actually, both types of effects had been in some way described previously by other investigators[2-5].

In order to clarify that complex and contradictory mechanism, a different model was designed. It consisted in the study of the immunological response to rat erythrocytes in mice injected previously with RNA extracted from the spleen of immune and normal mice.

Inbred mice of the BALB strain, 8–20 weeks old were used. These mice were immunized by an i.p. injection of a 50% suspension of fresh, washed rat red blood cells (RBC), from a highly inbred line, in 0.1 ml of phosphate buffered saline (pH 7.4). RNA was extracted from the spleen of immune and normal mice. The extraction of RNA was performed by the phenolic fractionation method described by GEORGIEV et al.[6] and it was obtained at room temperature in the first part of the procedure. The immune and normal RNA were diluted in 0.14 *M* sodium chloride and each animal received the RNA contained in one spleen (approximately 2 mg). The experimental mice received an RNA injection by i.p. route and 5 days later they were injected i.p. with 0.1 ml of 50% rat RBC in a buffered saline solution. 3 groups of animals were studied: (a) controls, (b) receiving immune RNA and (c) receiving normal RNA.

Serum samples were obtained by retro-orbital puncture[7] and hemagglutinins to rat erythrocytes were measured by a serial dilution procedure consisting of mixing equal volumes (0.02 ml) of the respective serum dilution and an 0.5% suspension of rat RBC.

During the 5 days which elapsed between the administration of immune RNA and the injection of rat RBC, no circulating hemagglutinins against rat RBC were detected. Hemagglutinins only appeared after the injection of rat RBC and the peak of antibody response was observed in normal controls at 6–8 days after this antigenic stimulus (Figure 1). At this time the groups of mice injected with RNA, both normal and immune, had a deep depression in the immunological response if compared with controls, the effect being greater in those animals receiving normal RNA (Table). The difference in titer between immune and normal RNA injected mice might be a measure of the immunological information carried by immune RNA. This interpretation is also reinforced by the following fact: when the immune RNA extraction was carried out at different periods of time after the immunization procedure in the donor animal, between 35–81 days, it was observed that (within the immune RNA injected group) the agglutination titer decreased significantly as the time from immunization to RNA extraction increased ($r = -0.51$, $p < 0.01$) (Figure 2). The immune RNA injected group never reached the response of the controls, probably because this RNA contained a high percentage of normal RNA which acted depressing the immunological response and thus counteracting the effect attributable to the information carried by immune RNA. The RNA injected contained 2% protein and 9% DNA. The influence of these 2 contaminants cannot be discarded, but the discussion is based on the assumption that the main effect is due to RNA.

These findings confirm the results reported by different authors and show that normal RNA has a strong immunosuppressive effect. It was known that normal RNA was able to inhibit the immunological information carried by rabbit lymphocytes if they were incubated with normal RNA[5]. Recently, it has been observed that dog kidneys perfused with normal RNA are better accepted than controls when grafted into homologous hosts[8]. In the

Hemagglutination titers in sera of mice treated with immune and normal RNA 8 days after the injection of rat RBC

| Treatment | $n$ | $\bar{x}$ | $P$ |
|---|---|---|---|
| Controls | 19 | 1125 | $< 0.001$ |
| Immune RNA | 24 | 487 | $< 0.01$ |
| Normal RNA | 21 | 278 | |

The means are the reciprocals of the hemagglutination titers. The immune RNA values were adjusted by a linear regression to a period of 35 days from immunization to RNA extraction (see text). $P$ values were obtained by the Student's $t$-test.

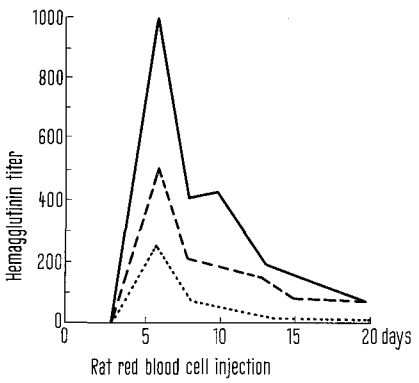


Fig. 1. Hemagglutinin titers after the injection of rat erythrocytes in control mice (—) and in mice pretreated with immune RNA (– – –) and normal RNA (····).

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present experiments it has been shown that normal RNA can equally produce its effect when administered to the host 5 days before the injection of the antigen, in this case rat RBC, indicating that it probably operates by depressing the immunological system rather than blocking the antigen itself (either RBC or kidney cells), which agrees with the results of ASHLEY et al.[3] and of AXELROD and MEI LOWE[4]. Previous authors have studied the inhibitory effect of normal RNA on the mechanism of immunity associated with tissue transplantation, the experiments herein described show that normal RNA also inhibits the humoral immunological system. This effect of normal RNA depressing both types of immunological response is difficult to explain; it acts even when the same RNA (immune RNA) carries immunological information. It can be concluded that: (a) normal spleen RNA acts as a biological inhibitor on the humoral side of the immune mechanism as well as on homograft rejection, and (b) RNA from sensitized spleen has two fractions: (1) a small one, carrying the information for the immunological response, which works in a different way from that of messenger RNA because no circulating antibodies are detected in the present conditions before the antigenic stimulus, and (2) a large one, which apparently acts depressing immunity as normal RNA[9,10].

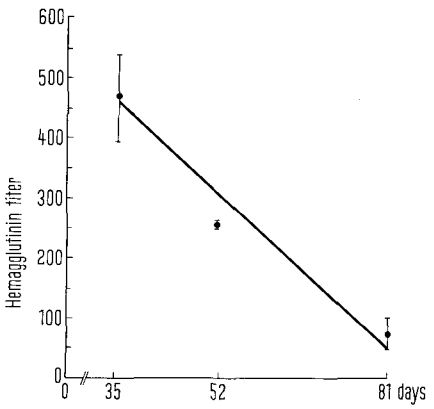


Fig. 2. Abscissa: Days elapsed from immunization to RNA extraction in donors of immune RNA. Ordinate: Hemagglutinin titer in mice receiving these same batches of immune RNA. The vertical lines represent the standard error of the mean.

*Resumen.* Se inmunizaron ratones BALB con glóbulos rojos de ratas de una línea endocriada. A estos ratones se les extrajo el ARN del bazo y se inyectó a otro grupo de animales de la misma cepa. Además, se inyectó el ARN proveniente de bazo de ratón normal a un segundo grupo de animales. Cinco días después de la inyección del ARN normal e inmune, ambos grupos, y un tercero, testigo, recibieron una inyección de glóbulos rojos de rata.

J. C. MORINI, M. V. LONDNER, MARÍA TERESA FONT and S. L. RABASA

*Instituto de Investigaciones Médicas and Facultad de Ciencias Médicas, Universidad de Rosario (Argentina), 20 January 1969.*

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## In vitro Study of C (Parafollicular) Cells of Dog Thyroid in Organ Culture

The presence of C, or parafollicular, cells in the mammalian thyroid has been investigated by various authors[1–4]. It has recently been shown[5,6] that the main function of these cells is the storage and secretion of calcitonin, the serum calcium lowering hormone[7]. As a response to hypercalcemia, C cells react in vivo by losing argyrophilia[3] and metachromasia[8] and by discharging the secretory granules[3,9].

The technique of in vitro organ culture has already been applied by numerous authors[10,11] to the study of follicular cells of normal and pathological thyroids, but, so far, no attention has been paid to the presence and behaviour of C cells.

We have cultured dog thyroids (from a total of 15 animals) in a chemically defined tissue culture medium (T.C. 199 'Wellcome') for 36–120 h, in atmosphere of 95% $O_2$ and 5% $CO_2$, using the method of CHEN[12].

To detect the presence of C cells, tissue blocks have subsequently been either (a) fixed in formol-Ca and cryostat sections stained with a silver method[13] for argyrophilic cells, or (b) fixed in glutaraldehyde- picric acid-acetate fixative and the sections stained with toluidine blue after mild acid hydrolysis[8], or (c) fixed in 3.12%

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