Surface Immunoglobulins of Circulating Lymphocytes in Mouse Plasmacytoma. II. The Influence of Plasmacytoma RNA on Surface Immunoglobulins of Lymphocytes

By Nirmala Bhoopalam, Vincent Yakulis, Nicolas Costea, and Paul Heller

Circulating lymphocytes of plasmacytoma-carrying BALB/c mice were found to lose their normal surface immunoglobulins; in their place surface structures characteristic of the specific plasmacytoma globulin were demonstrated by the immunocytoadhesion technique. These changes were experimentally reproduced by the incubation of normal BALB/c lymphocytes with an RNA preparation obtained by hot phenol extraction from the excised plasmacytomas. RNA treated by RNase was inactive, while DNase or trypsin had no inactivating effect. Lymphocytes, killed with heat or KCN, underwent no alteration of surface receptors following incubation with the tumor RNA. Plasmacytoma RNA, injected intraperitoneally into normal mice, also altered the reactivity of circulating lymphocytes. These observations suggest the possibility that this effect contributes to the functional impairment of the immune system in this disease.

In the preceding communication\(^1\) we reported that in BALB/c mice with plasmacytoma the proportion of circulating lymphocytes having normal immunoglobulin determinants on their surface diminished with the progression of the disease. Concomitant with this development, lymphocytes with surface structures characteristic of the plasmacytoma immunoglobulins were demonstrable. Absorption of these plasmacytoma globulins from the surrounding plasma was excluded by appropriate studies. The possibility was considered that this alteration was brought about by a product of the tumor other than the immunoglobulin itself.

It has been known for more than 10 yr that RNA extracts of antibody-producing lymphocytes are capable of transferring the information of antibody specificity to uncommitted cells.\(^2-9\) Recently, the studies of Bell and Dray\(^10,11\)
indicated that the allotypic specificity of lymphocytes can be altered by RNA obtained from lymphocytes of a different allotype, confirming the transforming capacity of the responsible RNA fraction. It appeared to be of interest to investigate the possibility that a similar phenomenon might be experimentally produced with plasmacytoma RNA and that the character of the surface immunoglobulins of normal BALB/c lymphocytes might be altered by RNA derived from the plasmacytoma. The results of these experiments indicate that incubation of normal BALB/c lymphocytes with the plasmacytoma RNA induces the same changes in the surface immunoglobulins as occur in animals carrying the tumor.1

MATERIALS AND METHODS

The preparation of the antisera, the methods of the immunocytoadhesion reaction of the coating of the erythrocytes with immunoglobulins, etc., were the same as in the accompanying communication.1 In some experiments designed to study the different classes of receptors on the lymphocyte surface, erythrocytes of chicken, in addition to those of BALB/c mice, were coated with the various immunoglobulins and used in a double, mixed antoglobulin reaction as previously described.12

Preparation of RNA

RNA was prepared from excised plasmacytomas of all variants under study (LPC-1, MOPC-195, MOPC-300, and MOPC-315) with the hot phenol extraction procedure of Scherrer and Darnell,13 as modified for the preparation of “immunogenic RNA” by Bell and Dray.10 The mean ratio of the absorbances at 260 and 280 μm for 25 RNA preparations was 2.0. These preparations were used in all transformation experiments in vitro and in vivo. In addition, an attempt was made to find an RNA fraction of the above preparation that would contain the transforming ability. Ultracentrifugation of the lyophilized RNA preparation was performed in AEC buffer (Na acetate, 10⁻² M; Na₂ EDTA, 10⁻³ M; NaCl 10⁻¹ M, pH 5.1) with a 5-40% sucrose gradient for 17 hr at 5°C and 25,000 rpm/min. This analysis gave a profile that did not reveal any observable breakdown of RNA. The usual three components with sedimentation coefficients of 4, 18, and 285 were obtained.

By filtration in C-200 Sephadex gel in 0.15 M NaCl, the RNA preparation was partitioned into three peaks. The exclusion peak presented 68% of the total amount of RNA and contained the transforming activity. Two other peaks with lower molecular weight contained 12% and 20% of the total RNA and were not active. Electrophoresis of the RNA preparation in polyacrylamide gel revealed three fractions, the largest being approximately 70% of the total; it moved only for a short distance anodically from the application area. The gel was stained by the method of Dahlberg et al.14 in which RNA, DNA, and protein are specifically stained. There were only minimal amounts of DNA or protein present in the preparation.

Incubation of Plasmacytoma RNA With Normal Lymphocytes

Aliquots of lymphocyte suspensions containing 5–10 × 10⁵ lymphocytes/0.1 ml were incubated with 1 mg of tumor RNA in Hank’s solutions for 30 min. Afterward, the cells were washed three times with BBS and used for the immunocytoadhesion reaction, as described in the accompanying communication.1 By varying the concentrations of RNA added to these aliquots of lymphocyte suspensions, the optimal amount of RNA effecting a change in the character of the surface structures of normal lymphocytes and the optimal incubation time was determined. The immunocytoadhesion reaction was also performed with RNA that had been treated with ribonuclease or deoxyribonuclease and with lymphocytes that had been treated with potassium cyanide or heat and no longer excluded trypan blue. One milligram of plasmacytoma RNA dissolved in 0.1 ml of Hank’s solution, pH
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Fig. 1. Percentage of receptor-carrying lymphocytes (RCL) after addition of varying amounts (µg) of LPC-1 plasmacytoma RNA to normal BALB/c lymphocytes. The Ig of this tumor belongs to γ2α class of mouse immunoglobulins.

7.4, was treated with varying concentrations of RNase (5X crystallized, Mann Research Labs, N. Y.) and DNase (Worthington Biochemical Corp., Freehold, N. J.) at 25°C and 37°C for 30 min.

In the in vivo experiments, BALB/c mice were injected with 1 mg of the RNA preparations intraperitoneally. Prior to the injection and periodically thereafter, peripheral lymphocytes were examined for surface immunoglobulins.

In addition to the tumor RNA, all the experiments described above were also performed with RNA obtained from normal spleen and liver of BALB/c mice.

RESULTS

In the in vitro experiments, the optimal amount of RNA effecting a change in surface immunoglobulins was 750 µg (Fig. 1), and the optimal incubation time was approximately 30 min (Fig. 2). In view of these results, 1 mg of RNA was used in all experiments. Following incubation, the proportion of normal lymphocytes having surface structures of normal immunoglobulins ("receptor-carrying lymphocytes," RCL) diminished from approximately 35% to 10–12%, but the proportions of lymphocytes reacting now with antisera to the plasmacytoma globulins increased from an insignificant amount to approximately 35–40% (Fig. 3). This phenomenon occurred in a specific manner depending on the tumor from which the RNA was obtained (Fig. 3). When the lymphocyte cultures were incubated with RNA preparations from two tumors at the same time, the majority of the lymphocytes had surface structures characteristic of both plasmacytoma globulins (Fig. 4). For the demonstration of these double receptors, the lymphocytes were sensitized with antisera to both
immunoglobulins, and mouse erythrocytes were coated with one immunoglobulin and chicken erythrocytes with the other. Mixed rosettes developed, indicating that the lymphocytes were capable of incorporating the RNA of both tumors simultaneously (Fig. 5).

The effect of the plasmacytoma RNA preparation from the LPC-1 tumor was tested with lymphocytes of other strains of mice. The percentage of lymphocytes carrying normal IgG structures decreased, while specific receptors on these lymphocytes were induced by the RNA preparation (Fig. 6).

Plasmacytoma RNA treated with RNAse did not produce a change in the

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**Fig. 3.** Receptor-carrying lymphocytes in vitro after addition of LPC-1 or MOPC-195 plasmacytoma RNA to normal BALB/c lymphocytes.

**Fig. 4.** Receptor-carrying lymphocytes in vitro after simultaneous addition of LPC-1 and MOPC-195 RNA to normal BALB/c lymphocytes.

**Fig. 5.** Mixed rosette formed by lymphocyte simultaneously exposed to RNA of LPC-1 and MOPC-195 plasmacytoma. For immunocytoadhesion reaction, lymphocytes were sensitized with antisera to both immunoglobulins, and chicken erythrocytes (nucleated) were coated with one immunoglobulin and mouse erythrocytes with the other.

**Fig. 6.** Receptor-carrying lymphocytes in vitro after addition of LPC-1 plasmacytoma RNA to lymphocytes of various mouse strains.
Table 1. Effect of RNase, KCN, and Heat on Surface Receptors Induced in Vitro by RNA of LPC-1

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*Receptor-carrying lymphocytes, RCL.

surface structure, but RNA treated with DNase or trypsin remained effective (Table 1). Heated lymphocytes or lymphocytes treated with KCN did not incorporate RNA and retained the original surface immunoglobulins (Table 1). RNA from liver and spleen also did not change the character of the surface structures.

The injection of 1 mg of LPC-1 RNA into normal BALB/c mice resulted in the reduction of IgG receptors from 38% to 17% and an increase of LPC-1 RCL from 2% to 43% (Fig. 7). The latter RCL gradually decreased within 2–3 wk and persisted at a lower level throughout the period of observation of 8 wk, while the normal IgG RCL first decreased and returned again to normal levels after 4 wk.

The possibility that the RNA preparation contained immunoglobulin was not only excluded by the ineffectiveness of RNA that had been treated with RNase, but also by the effectiveness of the RNA preparation treated with trypsin. Moreover, if RNA preparations were used in an attempt to inhibit specific hemagglutination reactions between immunoglobulin coated erythrocytes and antisera to these immunoglobulins, no inhibiting effect on the hemagglutination reaction was noted. Normal lymphocytes were also incubated with plasmacytoma globulins prior to their preparation for the immunocytoadhesion reaction. This preincubation had no influence on the outcome of this test, indicating that the immunoglobulins of the surrounding fluid are not absorbed to the lymphocytes and, therefore, are not responsible for the change in the immunocytoadhesion reaction.

Fig. 7. Receptor-carrying lymphocytes after injection of LPC-1 plasmacytoma RNA into normal BALB/c mice.
DISCUSSION

The experiments with plasmacytoma RNA have been stimulated by several reported observations during the last 10 yr, most recently and most impressively are those of Bell and Dray. These authors have shown that rabbit lymphocytes of one allotype are capable of producing antibodies of another allotype after incubation with "immunogenic" RNA from lymphocytes of this second allotype. In the present study, we have demonstrated the effect of the tumor RNA on the surface structures of the immunoglobulins of the circulating lymphocytes. The mode of synthesis of the surface immunoglobulins on normal lymphocytes probably differs from that of cytoplasmic immunoglobulins, since the surface immunoglobulins are not necessarily identical with those produced in the cytoplasm. Specific ribosomes near the membrane might be responsible for the synthesis of these surface immunoglobulins. It is conceivable that the converting RNA occupies such ribosomes normally utilized for the synthesis of surface immunoglobulins and reprograms them. No direct claim can be made as yet that the effect of RNA, as demonstrated in the present study, is an important component of the mechanism responsible for the diminished antigen responsiveness and reduced synthesis of normal immunoglobulins in mice with plasmacytoma and in patients with myeloma. Nevertheless, the results of the present study offer strong clues that this might, indeed, be the case. These studies, therefore, suggest that the functional impairment of the immune system in plasmacytoma is caused by cell products, among which a still biochemically undefined species of RNA is likely to be of great importance.

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REFERENCES

8. Adler, F. L., Fishman, M., and Dray, S.:


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