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Immunologic Response Restored by Macrophages in Mice with Immunosuppression Due to Normal RNA[1]

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The immunosuppressive effect of normal RNA has been reported previously by several authors (1–4), but its site of action is unknown. Recently, we (5) found that normal RNA depressed the immunologic system when injected 5 days before the antigen, confirming the results of others (1, 2). These experiments show that normal RNA effects both humoral and cellular immunity.

The purpose of this work is to determine where in the immunologic sequence RNA acts when administered before the antigen. If the immunologic sequence is assumed to be: antigen-macrophage-sensitized immunocyte (lymphocyte or plasma cell), it should be possible to restore the normal immunologic response after the administration of RNA by injecting these cells from isogenic normal donors if RNA acts on them. In the present experiment the action of normal macrophages has been investigated.

Inbred adult mice of the BALB strain were divided into 5 treatment groups: 1) controls, 2) normal RNA, 3) normal RNA plus macrophages, 4) normal RNA plus macrophages incubated with normal RNA and 5) normal macrophages. Each mouse in groups 2, 3, and 4 was injected with 2 mg normal spleen RNA intraperitoneally as previously described (5) and 5 days later all groups received intraperitoneally 0.1 ml of a 50% suspension of fresh, washed rat red blood cells (RBC) in a buffered saline solution. Normal macrophages (peritoneal cells which adhere to glass) and macrophages incubated with RNA were injected into the indicated groups intraperitoneally 1 day before the antigen (RBC). Each animal received approximately 4×10^5 cells. The peak hemagglutinin titer was used to evaluate the results.

Peritoneal cells from normal inbred BALB mice were collected by washing out the cavity with sterile Hanks-Simms solution 3 days after the injection of 1 ml of 2.5% starch gel. They were incubated with standard Hanks-Simms solution plus 5% rat serum and 5% hydrolysate lactalbumin at 37°C after 24 hr the supernatant was discarded. The cells adhering to the glass surface were removed by soft scraping, washed once, resuspended and counted in a hemocytometer. The viability, tested with 1% trypan red solution, was approximately 86%. Macrophages were incubated, as indicated, with normal spleen RNA at a concentration of 1.55 $\mu g/10^5$ cells at 37°C for 30 min in roller tubes. This treatment did not kill any cells.

Inhibition by normal RNA was reversed when exogenous macrophages were injected 4 days after RNA (Table I). Although the titer was slightly lower than controls the difference was not significant. On the other hand, animals receiving macrophages previously incubated with RNA had a titer similar to that of the group receiving RNA alone. Macrophages *per se* had no effect because animals receiving macrophages alone had the same titer as controls. It can be inferred that the immunosuppressive effect of RNA is exerted on the macrophages, perhaps blocking antigenic recognition and/or the transfer of information to immunocytes.

Animals belonging to groups 1, 2 and 3 were also used to study the secondary immune response. A second injection of rat RBC was administered about 30 days after the first stimulus. There was no difference between the group that received RNA plus macrophages and the controls. If both groups were pooled and compared to those receiving RNA alone a significant difference was obtained (Table I). The low hemagglutinin titer of the RNA-treated group shows that it had a weak store of information from the first antigenic stimulus, while the other two groups acquired good information against the antigen. The effect of RNA is then still evident in the secondary response, the immunologic memory of this group

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TABLE I

Immunologic response of mice treated with normal RNA plus macrophages and controls[a]

Treatments	Primary Response			Secondary Response		
	n	$\bar{x}$	P	n	$\bar{x}$	P
Controls	22	3491		15	23757	
Normal RNA + macrophages	21	2731	<0.001	14	20480	<0.01
Normal RNA	23	1102	<0.001	13	8585	
Normal RNA + macrophages (incubated with RNA)	10	1075				
Macrophages alone	6	2885				

[a] The means are the reciprocals of the peak hemagglutination titers after the first and second rat RBC injection. The data were transformed to $\log_2$ for statistical analysis. RNA was injected only once 5 days before the first antigenic stimulus. P values were obtained by the analysis of variance.

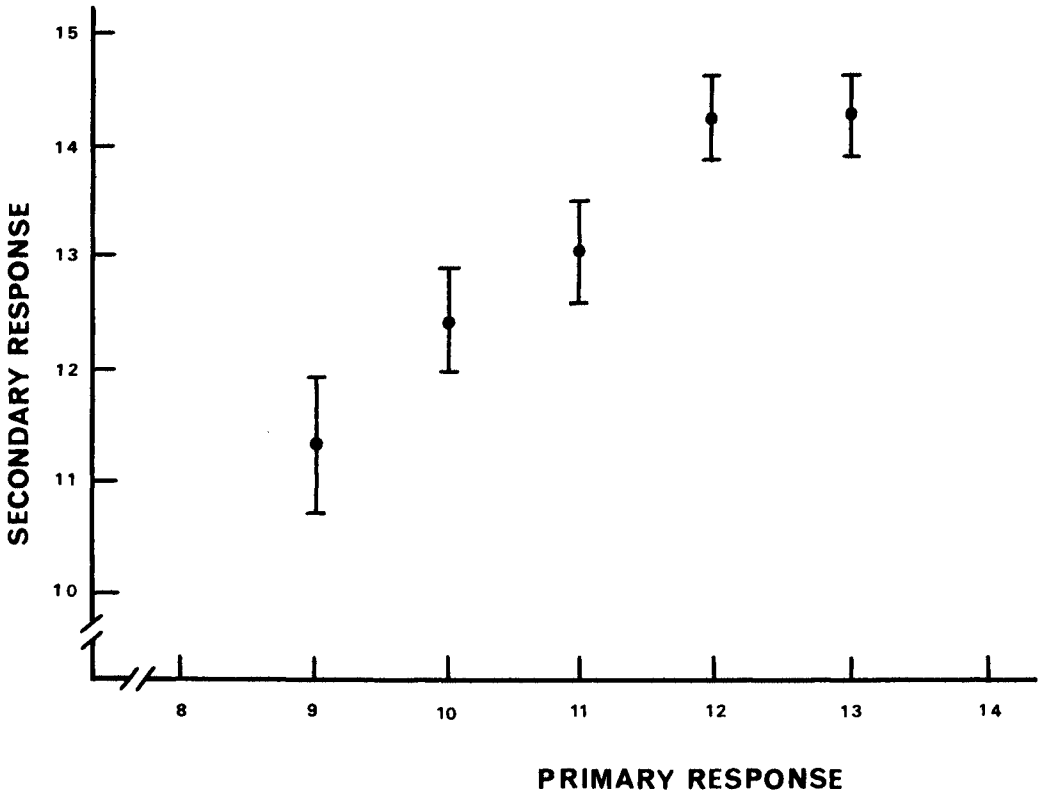

Figure 1. Correlation between primary and secondary responses in the same animal. The variable used both in the abscissa and in the ordinate is $\log_2$ of hemagglutinin titers. The vertical lines represent the standard error of the mean.

being less than that of normals. Moreover, macrophages seem to contribute to immunologic memory. It also follows that the titer reached in the secondary response depends on the quantity of immunologic memory accumulated during the first one, because those animals with a high response to the first stimulus had a high secondary response while a low secondary response usually came after a low first one. The correlation between the first and second immunologic response in the same animal was calculated (Fig. 1) and was highly significant ($r = 0.59$, $p < 0.001$, $n = 42$). This leads to the conclusion that immunologic memory is not only a qualitative but also a quantitative phenomenon.

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