INDUCTION OF AUTOHEMOLYSIN FORMATION IN SPLEEN CELL CULTURE BY "IMMUNOGENIC RNA"

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1. Introduction

When studying the mechanism of immune response, one of the fundamental questions that has to be answered is: why does an animal not produce antibodies against its own circulating "antigens"? Which one of the processes resulting in antibody production fails to work in this case?

Since the biochemical events from antigen uptake to antibody response are not well known and the circulating "self-antigen" has no immunogenic effect, an approach to this problem requires a system permitting the investigation of given stages of the immunization process.

Various reports describe the *in vivo* and *in vitro* induction of the synthesis of specific antibodies by RNA extracted from the spleen of immunized animals [1–3] or from macrophages after exposure *in vitro* to antigen [4–7] . These results provide an opportunity to investigate whether or not the inhibition of autoimmunization is accomplished before specific RNA is synthesized during immunization or *in vitro* during the antigen triggering period.

The experiments presented here suggest the possibility of initiating the formation of autohemolysin in guinea-pig spleen cell culture by RNA extracted from the spleen of rats immunized with guinea-pig red blood cells (GPBC) and by RNA extracted from rat spleen cells incubated with GPBC.

2. Methods

The guinea-pig spleen cells were cultured in a perfusion chamber [8] using Eagle's medium containing human serum. The animals used in these experiments were 400 g randomly bred male guinea-pigs. 5×10^7–1×10^8 spleen cells were dispersed on a glass filter immersed in 10 ml of medium. Usually 1×10^6 GPBC were present in the cultures. Generally the medium was changed daily. The hemolysin produced was measured in the centrifuged medium of spleen cells, red blood cells were added to the medium in 0.1 ml of 0.9% NaCl solution. The starting concentration of red blood cells was approximately 3×10^7 cell/ml. Red blood cells were counted after incubation at room temperature, for 4 hr, and hemolysis was expressed as percentage of hemolysed cells. The autohemolysin was tested by GPBC obtained from the same animals as the spleen cells of cultures. The specificity of hemolytic activity was controlled by simultaneous measurement of the hemolysis of sheep red blood cells (SRBC).

The "immunogenic RNA" was extracted according to Kruh [9] from the spleen of 200 g male Wistar rats 10 days after the primary or secondary stimulus with GPBC. In some experiments the "immunogenic RNA" was obtained by the same method from the spleen cells of 150 g male Wistar rats following the exposure of cells to GPBC at 2.5×10^7 spleen cell/ml and 6×10^6 GPBC/ml in a buffer solution [10] for one hr at 37°. RNA preparations showed an absorbance ratio (260/280) of 2.00 by MAK chromatography [11]. The RNA was added to the cultures in 0.2–0.5 ml volume. The "immunogenic RNA" itself did not show detectable hemolytic activity. RNase treatment of "immunogenic RNA" was carried out using 100 μg/ml of bovine pancreatic ribonuclease at 37° for 60 min.

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3. Results and discussion

Guinea-pig spleen cells produced autohemolysin on addition of "immunogenic RNA" obtained either from the spleen of immunized rats or from rat spleen cells incubated GPBC. The medium of control cells usually contained some hemolytic activity but this proved to be non-specific being equal with GPBC and SRBC. The "immunogenic RNA" evoked only the production of hemolysin specific to GPBC. An RNase treated extract failed to show the induction of autohemolysin production. RNA from normal rat spleen had no detectable effect (fig. 1). The induction effect seems to depend on the amount of RNA (fig. 2).

The GPBC used for the hemolysin test and the cultured spleen cells were taken from the same animal; moreover similar results were obtained with cells from 3–4 animals or from a single animal. It is therefore most improbable that the antigenic differences between GPBC from different guinea-pigs are responsible for the observed autohemolysin production. The autohemolysin induction cannot be due only to the presence of contaminating antigen or antigenic fragments in the "immunogenic RNA", because RNase treatment completely abolished induction and the GPBC alone were not effective.

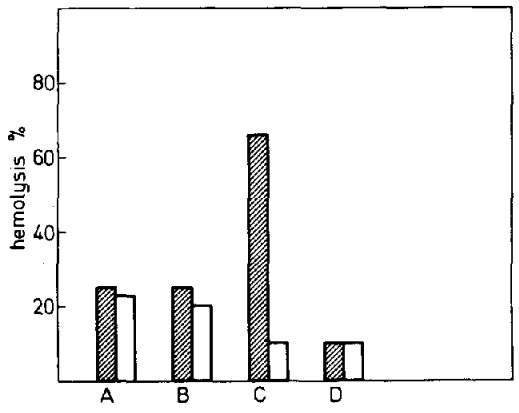

Fig. 1. The effect of "immunogenic RNA" on the production of autohemolysin in guinea-pig spleen cell culture on the second day. The cultures contained 5×10^7 spleen cells and 1×10^6 guinea-pig red blood cells. The hemolysis was measured with guinea-pig red blood cells (hatched columns), and sheep red blood cells (open columns) at room temperature, after four hr in the centrifuged medium of cultures. The hemolysis was expressed in percentage of hemolysed cells. (A) spleen cells, guinea-pig red blood cells; (B) 300 μg normal rat spleen RNA; (C) 300 μg "immunogenic RNA"; (D) 300 μg "immunogenic RNA" treated with RNase.

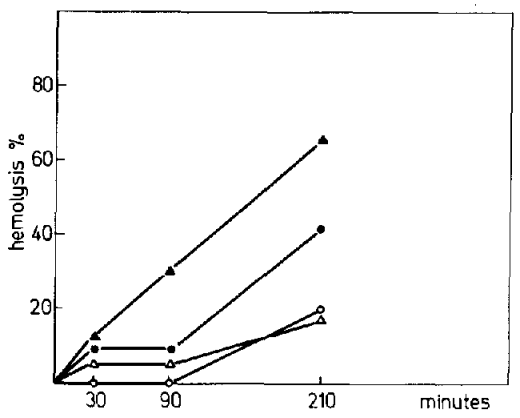

Fig. 2. The effect of the amount of "immunogenic RNA" on the induction of autohemolysin production in guinea-pig spleen cell culture containing 1×10^8 spleen cells and 1×10^6 guinea-pig red blood cells. The hemolysis was measured at time intervals shown. Sterile medium: ○—○; spleen cells alone: △—△; 300 μg "immunogenic RNA": ●—●; 1200 μg "immunogenic RNA": ▲—▲.

The possibility cannot be excluded that the autohemolysin altered during immunization could have been induced by GPBC. These results suggest that the RNA required for production of autoantibody is absent from spleen cells or present in inactive form. Thus processes preceding translation from RNA to proteins appear responsible for the absence of autoantibody responses to red blood cells.

The results of preliminary experiments on *in vivo* induction of autohemolysin by "immunogenic RNA" are consistent with the *in vitro* results. The high amount of antigen in the system studied does inhibit the detectable production of autoantibody in the presence of "immunogenic RNA".

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