Distribution of Antigen Fragments of IgG Among the Nucleotides of RNA

It is known that when antigen penetrates into a macrophage it is broken down into fragments with a molecular weight of 5,000, which complex with RNA of the macrophage. Similar complexes are found in the circulation[1], and in the lymph nodes and spleen[2] 5 h after i.v. injection of bovine serum albumin marked with ^{35}S. 85% of the original radioactivity is found bound to the RNA-antigen complex. This complex is necessary for the formation of antibody and takes part in the amnestic response[3].

The purpose of this communication is to give evidence that antigenic fragments are associated with certain nucleotides of RNA under conditions of antigenic stimulation using IgG as antigen.

Materials and methods. Guinea-pigs weighing 300–400 g were used. Antigen consisted of crystaline human serum γ-globulin (IgG) from Koch Light. 5 days after immunization of guinea-pigs with 20 mg/ml IgG, they were killed and RNA was isolated from the spleens according to the method of ADLER et al.[1]. Controls were made in the same manner using non-immunized guinea-pigs. In other experiments, the spleens from normal guinea-pigs were homogenized and then incubated for 2 h at 37 °C with 20 mg/ml IgG and then RNA was isolated. Controls were prepared by the same procedure but lacking IgG.

Table I. Serological Activity of nucleotides isolated from RNA of the spleen of guinea-pigs immunized against human IgG

Nucleotides[b]	Concentration (M)	Inhibition of hemolysis[a] (volume of antigen solution)		
		0.05 ml	0.10 ml	0.20 ml
AMP	0.6×10^{-5}	+++	++++	++++
GMP	1.5×10^{-3}	++	++++	++++
CMP	2.0×10^{-3}	+	++++	++++
UMP	2.1×10^{-2}	—	—	—

[a] ++++ complete inhibition; +++ 75% inhibition; ++ 50% inhibition; + 25% inhibition; — no inhibition. [b] AMP adenosine monophosphate; GMP guanosine monophosphate; CMP cytidine monophosphate; UMP uridine monophosphate.

Table II. Serological activity of nucleotides isolated from RNA of spleen homogenates incubated with IgG

Nucleotides	Concentration (M)	Inhibition of hemolysis[a] (volume of antigen solution)		
		0.05 ml	0.10 ml	0.20 ml
AMP	1.1×10^{-4}	+++	++++	++++
GMP	2.2×10^{-3}	++	++++	++++
CMP	2.0×10^{-3}	++	++++	++++
UMP	2.7×10^{-2}	—	—	—

[a] See Table I for explanation.

A control for non-antibody-producing cells was made by using monolayers of primary pig kidney tissue cultures in the presence and absence of antigen. RNA hydrolysis and the separation and measurement of nucleotides was made according to the method of KATZ and COMB[4]. Serological determinations were made by the microcomplement fixation test[5]. Sheep immune serum against human IgG was received from the Institute of Microbiology, Sofia.

Results. The result of serological activity of nucleotides isolated from RNA of the spleens of guinea-pigs immunized against human IgG are shown in Table I.

From Table I it is seen that 0.1 ml AMP ($0.6\times10^{-5}M$) gives complete inhibition of hemolysis, UMP in doses 3000 times more concentrated does not inhibit hemolysis. The higher concentrations of GMP and CMP necessary to cause inhibition of hemolysis show that the antigen determinants of IgG are bound predominantly with AMP and in smaller quantities with GMP and CMP.

RNA isolated from primary pig kidney cell cultures in the presence or absence of IgG does not inhibit hemolysis. RNA isolated from non-immunized control spleens also gives negative results.

The results of experiments in which RNA was isolated from spleen homogenates incubated with IgG, are given in Table II. The results of in vitro experiments show that the determinant groups of IgG are distributed among the nucleotides in the same manner as in vivo experiments.

Discussion. According to ROELANTS and GOODMAN[6], the formation of the RNA-antigen complex is independent of cell integrity and can occur in an in vitro system. Our results with RNA isolated from spleen homogenates from pig kidney cells are in disagreement with this finding, because binding of antigenic determinants with RNA occurs only in antigen processing cells.

The higher serological activities of AMP and GMP, according to our results, show that the antigenic fragments are bound predominantly with the mononucleotides of purine bases, which have a higher electrostatic charge.

According to HALAC et al.[7] and NACHKOV et al.[5] the RNA of antigen stimulated macrophages is richer in purines and poorer in pyrimidines than is the RNA of non-stimulated cells. We suggest that the function of the higher purine content of RNA in antigen stimulated macrophages and the association of antigenic determinants predominantly with the purines is to give a larger quantity of immunogenic material.

Zusammenfassung. Feststellung, dass die Antigen-Determinanten meistens mit Purin-Mononukleotiden (AMP und GMP) verbunden sind, was auf eine charakteristische und spezifische Eigenschaft der Antigen-«processing»-Zelle zurückgeführt wird.

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