EVIDENCE FOR AN UNUSUAL IMMUNOLOGICAL RESPONSE TO ISOPENTENYLADENOSINE IN RABBITS

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1. Introduction

N^6-($\triangle^2$-Isopentenyl)adenosine (iA), apart from its little known function in certain species of tRNA from a variety of organisms, is also known to be a potent cytokinin [1]. This hyper-modified nucleoside has further been reported to have a bewildering variety of biological effects [1–6]. Among other things, iA has been shown to be immunosuppressive [5].

In the course of our investigations on nucleic acid-reactive antibodies [7–9], we raised anti-iA antibodies in rabbits with the intention of using them in tRNA research. For immunization, iA was coupled to bovine albumin by two methods. One involved the 'periodate procedure' of Erlanger and Beiser [10]; The other involved the synthesis of isopentenyladenosine 5′,3′-(2′)-diphosphate (piAp) and its subsequent coupling to albumin by a water-soluble carbodiimide (quoted in ref. [7]). During the preparation of gamma-globulins from sera by salt precipitation [11], it was observed that anti-iA and anti-piAp sera gave unusually bulky precipitates. This observation, coupled to the exceptionally high titres of precipitable antibodies in these sera and the known multifunctional nature of iA prompted us to undertake an analysis of the protein distribution of these sera in comparison to other anti-nucleotide sera and normal rabbit sera. The results described in this communication show that both the iA-albumin and piAp-albumin conjugates elicited an unusually strong antibody response accompanied by a marked hyperglobulinemia.

2. Materials and methods

iA was purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. piAp was synthesized by phosphorylation of iA by a procedure to be described elsewhere. iA-albumin and piAp-albumin were prepared according to the published procedures [7,10]. 1 to 1.5 mg of the appropriate conjugate was injected at a time in complete Freund's adjuvant into random-bred albino rabbits (two animals for each conjugate). Immunologic procedures like injection and bleeding schedules, quantitative precipitin reactions etc. were according to the general methods described earlier [7]. The preparation of [^{3}H]iA has been described elsewhere [8]. Gel-filtration of sera on Sephadex G-200 was carried out according to the method of Flodin and Killander [12]. Cellulose acetate-membrane electrophoresis of serum samples was performed by the method of Kohn [13], except that Amido Black 10B was used for staining.

3. Results

Table 1 gives the titre ranges of precipitable antibodies present in the sera of rabbits immunized with various nucleoside– of nucleotide–albumin conjugates. It was noticed early during immunization (after the second injection) that anti-iA and anti-piAp sera gave very heavy precipitin bands in agar diffusion experiments. Quantitative precipitin reactions showed

Table 1
Antibody content of anti-nucleoside and anti-nucleotide sera

Antiserum	Antigen	Antibody precipitable* mg/ml of serum
Anti-iA	iA–albumin	11–16
Anti-piAp	piAp–albumin	10–15
Anti-dAMP	dAMP-albumin	1–3.5
Anti-dCMP	dCMP–albumin	< 5

* Quantitative precipitin reactions with the respective antigens were performed as mentioned in Materials and methods. The results represent the range of antibody content during the course of immunization. At least two rabbits were immunized with each antigen.

that the antibody content of even early sera exceeded 10 mg/ml. This is in contrast with the sera derived from rabbits under immunization with the common nucleotide–albumin conjugates, which showed weak titres at the beginning and attained maximal antibody levels of 5 mg/ml or less only after several injections.

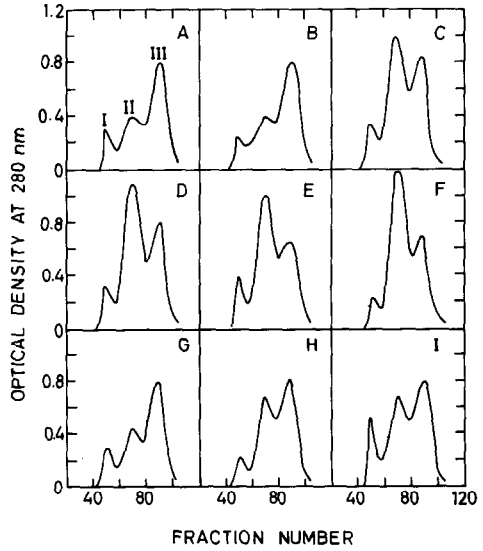

Fig. 1. Sephadex G-200 chromatography of some normal and immune sera. 0.4 ml of serum was loaded on a column of Sephadex G-200 (2 × 50 cm) equilibrated with Tris-buffered saline containing 0.1 M Tris–HCl (pH 7.5), 0.14 M NaCl and 0.02% NaN_3. Fractions (1.2 ml) were monitored for UV absorption. (A,B) Normal sera collected before immunization (rabbits R-6,R-8); (C,D) anti-iA sera (R-6,R-7); (E,F) anti-piAp sera (R-8,R-9); (G,H) anti-dAMP sera (R-1, R-5); (I) anti-dCMP serum (R-41).

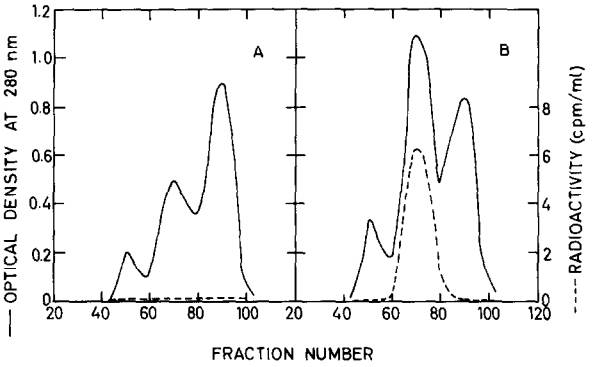

Fig. 2. Distribution of [^{3}H]iA-binding activity in an anti-iA serum. 0.4 ml of serum was incubated with 2 µl of a stock solution of [^{3}H]iA containing a total of 300 000 cpm (specific activity: 2000 cpm/pmol) for 30 min at 37°C and then chromatographed on Sephadex G-200 as described in legend for fig. 1. Aliquots (0.2 ml) from each fraction were dried on a filter paper strip (2 × 4 cm) and the radioactivity measured after placing the strip in a scintillation vial containing 10 ml of a 0.5% solution of PPO in toluene. A, Normal serum; B, anti-iA serum.

Fig. 1 shows some representative patterns obtained by gel-filtration of various normal and immune sera. Fractionation of serum on Sephadex G-200 gives three peaks, peak I containing the macroglobulins while the globulins make up most of peak II; Peak III is largely made up of albumin [14]. It is apparent from the gel-filtration patterns (fig. 1) that there is a marked increase in peak II (i.e. the globulin peak) in the case of anti-iA and anti-piAp sera. A comparative study of 25 serum samples from a total of 14 rabbits under similar immunization schedules with various nucleoside– and nucleotide–albumin conjugates revealed that only the anti-iA and anti-piAp sera showed a consistent and marked increase in peak II. Such hyperglobulinemia could be noticed in the sera collected after the second injection. On the other hand, sera of rabbits under immunization with other nucleotide–albumin conjugates (e.g. dAMP–albumin and dCMP–albumin) the increase in the globulin content was either marginal or was moderate only after several booster injections. In almost all cases the globulin content was lower than that of albumin.

In order to check whether the antibodies belonged to the 19 S or 7 S group, the anti-iA sera were incubated with [^{3}H]iA and then gel-filtered (cf. [14]). The distribution of the radioactivity (fig. 2) clearly

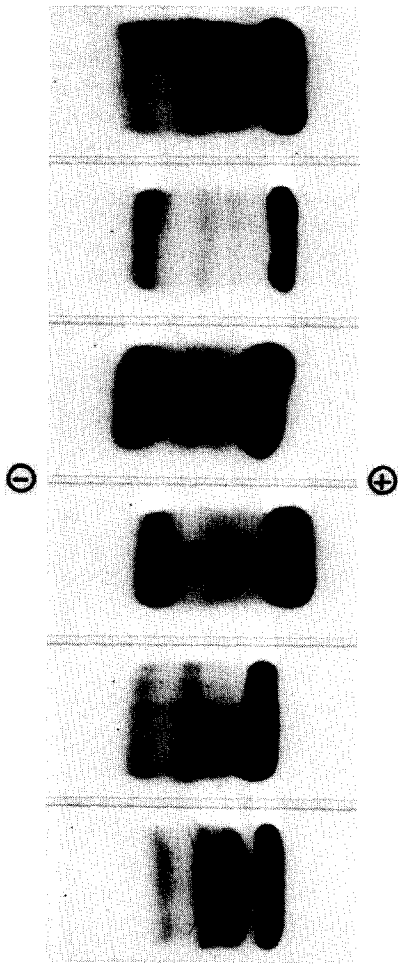

Fig. 3. Cellulose acetate-membrane electrophoresis of normal and immune sera. Procedure as mentioned in Materials and methods. From the top, 1, represents normal rabbit serum; 2 and 3, anti-iA sera; 4, anti-piAp serum; 5, anti-dAMP serum; 6, anti-dCMP serum.

showed that all the iA-reactive antibodies were to be found in peak II.

Fig. 3 shows the cellulose acetate-membrane electrophoresis patterns of normal and some representative immune sera. It can be seen that in the case of the anti-iA and anti-piAp sera there is a marked increase in the gammaglobulin content as compared to either normal sera or anti-dAMP or anti-dCMP sera.

4. Discussion

It is important to note that under almost identical conditions only those rabbits that were injected with conjugates bearing the isopentenyladenosine moiety as the haptenic group responded with such unusual intensity. Both the high titres of precipitable antibodies as well as the marked hyperglobulinemia were evident early during the course of immunization. In comparison, neither such high antibody levels nor elevation in globulin content were noticeable in the other immune sera. It would therefore appear that the intense immunologic stimulation is a specific effect of isopentenyladenosine. This is curious, since the free nucleoside itself has been reported to be antiproliferative as well as immunosuppressive [5,6]. The antibodies themselves are highly specific for iA and piAp, very little being directed against the carrier protein (see, e.g., ref. [9]).

Purine–protein conjugates have been shown to have specific tumor-regressing properties and this effect has been correlated to their antigenicity by Lachman and Cohen [15]. It has been suggested by these authors that the action of purine–protein conjugates lies in their capacity to stimulate a weak and depressed immune mechanism in the host. The intense antigenic stimulation caused by iA– and piAp–albumin conjugates may be of special significance in this context. Experiments to test the effectiveness of these conjugates in regressing Yoshida Ascites tumor in rats are in progress in our laboratory.

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