EFFECT OF N^6-(Δ^2-ISOPENTENYL)-ADENOSINE ON THYMUS AND SPLEEN IN RELATION TO ANTIBODY FORMATION IN MICE

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1. Introduction

N^6-(Δ^2-Isopentenyl)-adenosine (IPA), a biologically potent substance with cytokinin activity has been isolated from tRNA of a variety of plants, microorganisms and mammalian tissues [1]. Recent studies have demonstrated that IPA is able to inhibit blast transformation induced by phytohaemagglutin (PHA) in human and rat lymphocyte cultures. In addition, this substance inhibits the incorporation of ^{3}H-uridine and ^{3}H-thymidine, respectively, in PHA-stimulated rat spleen cells and the immediate cutaneous hypersensitivity in vivo [2]. In contrast, Braun and Nakano [3] have reported that IPA has no effect on plaque forming cells (PCF) in the spleens of mice immunized with sheep red blood cells (SRBC) and inhibits only the polyriboadenylic-polyribouridylic acid enhanced PFC-response to SRBC.

It was therefore of interest to explore why IPA has no effect on the normal immune response to SRBC whereas it suppresses immediate hypersensitivity in vivo. The data presented in this paper show that IPA does inhibit 19 S hemolysin forming cell responses in mice immunized with optimal and suboptimal doses of SRBC. ^{3}H-Leucine and ^{14}C-uridine incorporation showed that IPA interferes with the mechanism of cellular RNA and protein synthesis in thymus cells but not in spleen cells from SRBC-immunized mice and also inhibits macrophage migration.

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2. Materials and methods

2.1. *Immunization of mice and IPA treatment*

4–5 week-old mice (NMRI/HAN) were inoculated with graded amounts of SRBC intraperitoneally. Other groups of mice were treated with IPA (100 mg/kg) given i.p. at various time intervals.

2.2. *Assay for 19 S hemolysin forming cells (PFC)*

Mice were killed 96 hr after immunization by decapitation. Spleenic 19 S PFC response was determined according to Jerne et al. [4]. For statistical analysis the Wilcoxon test [5] was used.

2.3. *^{3}H-Leucine and ^{14}C-uridine incorporation*

Thymus and spleen from non-immunized mice and immunized mice (2.0×10^7 SRBC) were pooled separately. Thymus and portions of spleen were pooled in the same way from IPA treated immunized mice and cell suspensions prepared.

Thymus tissue was teased with forceps and shaken for 1 hr at 4° in Krebs-Henseleit-buffer of pH 7.4 (KHB) in order to prepare cell suspensions. Spleen cell suspensions in KHB were prepared by repeated withdrawal of teased spleen with a sterile plastic syringe and final filtration through a fine sieve. In both cases concentration was adjusted to 1.0×10^7 cells/ml in KHB. To each 2 ml of thymus and spleen cell suspension 1 μCi of ^{3}H-leucine and ^{14}C-uridine (The Radiochemical Centre, Amersham) were added separately. Incubation was continued for one hour at 37°. The reaction was stopped by the addition of 0.5 ml of 1% inactive leucine and uridine respectively and precipitation by 5% trichloroacetic acid (TCA)

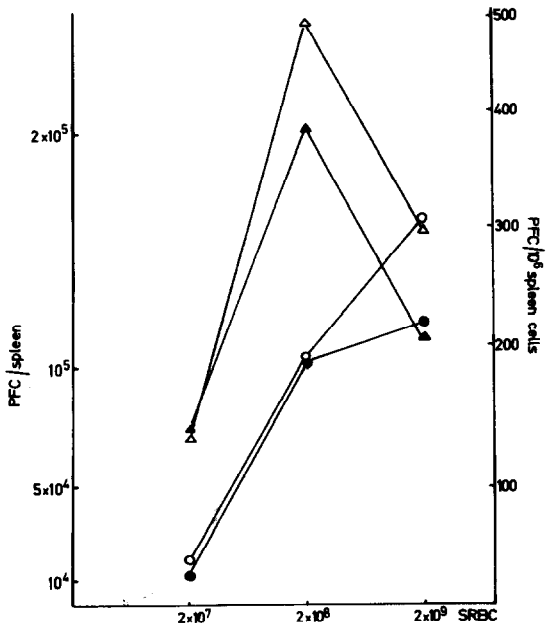

Fig. 1. Antigen dose dependent immunosuppressive effect of IPA. 100 mg/kg/day IPA treatment for seven days (three days before untill three days after immunization); ▲, PFC/spleen in control group; △, PFC/1.0 × 10^6 spleen cells in control group; ●, PFC/spleen in IPA treated group; ○, PFC/1.0 × 10^6 spleen cells in IPA treated group.

final concentration). TCA-precipitable material was washed in each case three times after homogenization. The acid-insoluble material was then adjusted to 4 ml in 5% TCA and an aliquot was measured for ^{3}H and ^{14}C-cpm in Tri-Carb-Scintillation-Spectrometer (Packard). The remaining sediment was used for RNA isolation and measurement [6, 7]. Protein was determined according to Lowry et al. [8]. Radioactivity was measured in isolated fractions in order to express ^{14}C-uridine/mg RNA and ^{3}H-leucine/mg protein.

2.4. *Macrophage migration*

The method used was similar to that described by David et al. [9] except that the peritoneal cell concentration before centrifugation of cells in capillary tubes (1.2 mm in diameter, 80 mm long) was 60 × 10^6/ml medium. The capacity of the Mackaness-type chambers was 1.25 ml. Control chambers contained only medium whereas other chambers were filled with 1 to 1000 μg IPA/ml medium. The area of migration was determined with a planimeter after 48 hr incubation at 37°.

3. Results and discussion

When IPA (100 mg/kg) is given over a period of seven days it reduces the number of PFC per spleen and per 1.0 × 10^6 nucleated spleen cells in mice immunized with SRBC. However, this effect could be demonstrated only at optimal (2.0 × 10^8) and suboptimal (2.0 × 10^7) SRBC doses. IPA has no significant effect on PFC when mice are immunized with high antigen dose (fig. 1).

Braun and Nakano [3] demonstrated that IPA has no effect on PFC-response measured 48 hr after the SRBC inoculation. This discrepancy may be due to the different experimental conditions (the antigen dose, the route of antigen inoculation, the time of IPA treatment and the time of assaying PFC-response) used by these authors.

It is evident from the result presented here that immunosuppressive activity of IPA depends mainly upon the time of application in relation to the day of immunization (fig. 2). The PFC response to SRBC is only inhibited when IPA administration precedes antigen treatment. When IPA is given after immunization no effect on the PFC response is noticed. However, in both cases a significant involution of thymus without any alteration in the weight of spleen was observed.

In our system IPA has no significant effect on the incorporation of ^{3}H-leucine and ^{14}C-uridine into protein and RNA of SRBC-stimulated spleen cells. However, IPA exerts a marked inhibitory effect on the incorporation of ^{3}H-leucine and ^{14}C-uridine in SRBC-stimulated thymus cells (table 1) so that IPA or its metabolites may exert their effect primarily on thymus-cell-mediated responses. In addition, as evident from table 2 IPA also inhibits macrophage migration in vitro and a similar in vivo effect might diminish immunologically mediated inflammatory responses.

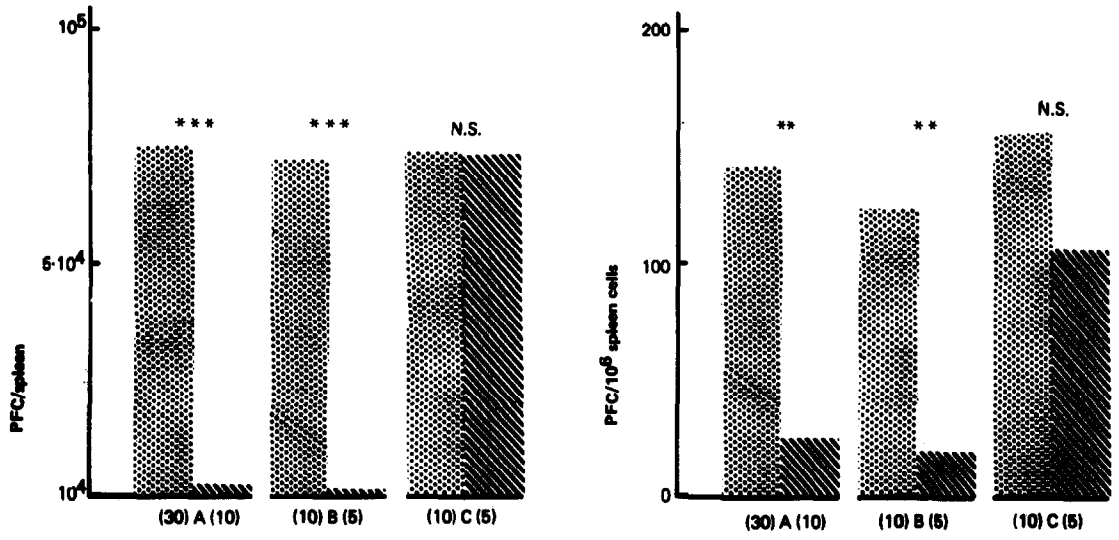

Fig. 2. Effect of timing of IPA treatment on PFC-response. Mice were immunized with 2.0×10^7 SRBC. Figures in brackets refer to number of mice; (A) 100 mg/kg/day IPA treatment for seven days (three days before till three days after immunization); (B) 100 mg/kg/day IPA treatment for four days (three days before and the day of immunization); (C) 100 mg/kg/day IPA treatment for four days (beginning on the day of immunization). Control group; IPA treated group. N.S., not significant; * $p = 0.1$; ** $p = 0.05$; *** $p = 0.02$.

Table 1

Effect of IPA on ^{3}H-leucine and ^{14}C-uridine incorporation in thymus and spleen cells of non-immunized, immunized and IPA treated immunized mice.

Group	Radioactivity (cpm)			
	Thymus		Spleen	
	^{3}H-Leucine/mg protein	^{14}C-Uridine/mg RNA	^{3}H-Leucine/mg protein	^{14}C-Uridine/mg RNA
Control	1,090	9,250	1,225	4,970
Immunized	4,410	23,800	1,652	14,340
IPA treated and immunized	1,761	14,200	2,066	13,210

Mice were immunized with 2.0×10^7 SRBC.
IPA treatment: 100 mg/kg i.p. for four days (three days before and the day of immunization).

Table 2
Effect of IPA on macrophage migration.

Substance (g/ml)	Area of migration* (mm^2 ± S.E.M.)	% inhibition of migration
Control	62.0 ± 1.8	
IPA		
1×10^{-6}	55.8 ± 2.2	10.0
1×10^{-5}	47.0 ± 2.3	24.2
1×10^{-4}	25.4 ± 1.1	59.0
1×10^{-3}	complete inhibition	

* Areas of migration were measured by planimetry after 48 hr of incubation.

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