gestive enzymes will be uncovered by further studies of insect-microbial associations and of the utilization of refractory plant materials by arthropods.

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6. Most of the larvae died before the end of the first week when fed symbiont-free balsam fir chips. It was necessary to pool the surviving larvae to obtain sufficient material for a single series of enzyme assays.
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10. Larval midguts, in groups of 10 to 20, were homogenized and centrifuged. The extract was desalted on a Pharmacia PD-10 column and soluble proteins were dialyzed against 50 m*M* acetate, *p*H 5.0. Cell-free fungal culture fluid was concentrated in a Pellicon Cassette (Millipore). Proteins were precipitated with ethanol at −20°C and dialyzed against acetate buffer, *p*H 5.0. Larval and fungal extracts were treated similarly thereafter. Cellulases and xylanases were fractionated on a column (2.6 by 40 cm) of DEAE-Sepharose CL6B equilibrated with 5 m*M* acetate buffer, *p*H 5.0, and eluted with a 0 to 500 m*M* linear NaCl gradient. Four major peaks of xylanase activity were eluted from the column at concentrations of 35 m*M*, 80 m*M*, 165 m*M*, and 345 m*M* NaCl. Two major cellulases emerged from the column at concentrations of 295 m*M* and 370 m*M* NaCl. Active fractions were further fractionated on a column (2.6 by 40 cm) of Sephadex G75 eluted with 50 m*M* acetate buffer, *p*H 5.0. Isoelectric focusing on an ion exchanger (chromatofocusing) was performed on a column (1 by 40 cm) of Polybuffer Exchanger 94 (Pharmacia) equilibrated with 25 m*M* imidazole HCl, *p*H 7.4, and eluted with 0.0094 m*M* Polybuffer 74 (Pharmacia) per *p*H unit per milliliter, *p*H 4.
11. The second cellulase fraction did not elute from the column, since the *p*H gradient was set to run from *p*H 7 to 4. This cellulase was eluted with an NaCl solution. That the *p*I values of the proteins in this fraction are less than *p*H 4 was confirmed by polyacrylamide gel isofocusing (Fig. 1).
12. Analytical isofocusing was performed on 0.1-mm polyacrylamide gels (Servalyt Precotes, Serva Fine Biochemicals) with a nominal *p*H range of 3 to 6. Focusing was performed for 2000 volthours (final field strength of 100 V/cm) at 10°C in a Desaga flatbed apparatus. Gels, fixed in 20 percent trichloroacetic acid, were stained with silver nitrate [C. R. Merril, D. Goldman, S. A. Sedman, M. H. Ebert, *Science* **211**, 1437 (1981)]. Amyloglucosidase, glucose oxidase, soybean trypsin inhibitor, β-lactoglobulin A, and bovine carbonic anhydrase B (Serva Fine Biochemicals) were used as *p*I marker proteins.
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21. We thank A. Sakai, R. VandeKopple, G. Keevil, and E. Weatherbee for field assistance, the University of Michigan Biological Station and the Matthaei Botanical Gardens for use of their facilities, and the National Science Foundation for grants PCM-78-22733 and PCM-82-03537 to M.M.M.

8 November 1982; revised 4 February 1983

Is Thymosin Action Mediated by Prostaglandin Release?

Abstract. *Treatment of spleen cells derived from adult thymectomized mice with thymosin fraction 5 resulted in a rapid and dose-dependent stimulation of the release of immunoreactive prostaglandin E_2. The release of prostaglandin E_2 was associated with induction of theta antigen and was totally inhibited by indomethacin. In contrast, prostaglandin E_2 release from spleen cells from intact donors was inhibited by treatment with fraction 5. The data support the concept that prostaglandin E_2 mediates the effects of thymosin fraction 5 on lymphocytes.*

Prostaglandins have been implicated as possible mediators of the biological activity of thymic factors. Studies in our laboratory showed that an analog of prostaglandin E_2 (PGE_2) was able to mimic the action of thymic factors in the induction of theta antigen and in the appearance of serum thymic-like activity (STA) when it was given to adult thymectomized mice (*1, 2*). Moreover, the action of thymosin on theta antigen and on STA appearance was completely abrogated by the administration of indomethacin, a potent inhibitor of prostaglandin synthetase. In the experiments reported here we investigated the mode of action of thymosin on lymphocytes and examined the relation between thymosin and prostaglandins. Our results show that thymosin induces an early and dose-dependent release of high concentrations of PGE_2 by lymphocytes collected from thymectomized mice.

For these experiments we used 4- to 8-week-old male C57BL/6J mice obtained from ENEA-Casaccia, Rome, Italy. Spleen cells from thymectomized or intact mice were incubated with 100 μg of thymosin fraction 5 (Fr5) (Hoffmann–La Roche, Nutley, New Jersey) with or without indomethacin. At various time intervals, the amount of PGE released was measured by using the radioimmunoassay described by Jaffe *et al.* (*3*). As early as 5 minutes after incubation, spleen cells obtained from thymectomized mice and treated with Fr5 released markedly higher concentrations of PGE_2 than untreated spleen cells or spleen cells from thymus-intact mice (Fig. 1A). This difference persisted at least for 1 hour. Conversely, spleen cells from intact donors showed a slight inhibition of the PGE release after exposure to Fr5. Indomethacin inhibited the release both from Fr5-stimulated spleen cells from thymectomized mice and from intact donors.

In a second experiment, spleen cells from thymectomized and intact mice were separated on sodium metrizoate–Ficoll solution (Lymphoprep) and incubated with various concentrations of Fr5. After 15 minutes (that is, at the time of full PGE release) specific PGE_2 release was measured by radioimmunoassay. Thymosin Fr5 increased the PGE_2 release by lymphocytes from thymectomized mice in a dose-dependent fashion (Fig. 1B). Conversely, in normal splenic lymphocytes, small amounts of Fr5 stimulated a slight PGE_2 release, whereas high concentrations of the factor (10 to 100 μg/ml) inhibited the spontaneous release of PGE_2. Furthermore, most of the PGE released by splenocytes belonged to the PGE series. Treatment with indomethacin inhibited PGE_2 release.

Figure 2 shows the relation between the induction of theta-antigen in vitro and the release of PGE_2 by lymphocytes derived from thymectomized mice and incubated with various amounts of Fr5. The presence of theta antigen was evaluated by measuring the concentrations of azathioprine (AZ) required for inhibiting splenic, spontaneous rosette-forming cells, according to a modification of Bach's technique (*4*). Theta-positive cells were defined as those in which rosetting was inhibited by 1.5 μg of AZ. High concentrations of Fr5 (100 μg/ml) induced the presence of theta antigen and stimulated the greatest release of PGE_2, whereas low levels of the factor (1 to 5 μg/ml) were unable to induce theta antigen and produced only a limited amount of PGE_2.

Our results show that Fr5 stimulates an early and consistent release of PGE from splenic lymphocytes of thymectomized mice. Whereas 100 μg of Fr5 per milliliter was effective in inducing a considerable release of PGE_2 in lymphocytes from thymectomized mice, the same concentration was ineffective in normal lymphocytes; PGE_2 release from normal splenocytes was stimulated by 5 to 10 μg of Fr5 per milliliter and inhibited by higher concentrations. These different responses may be related to the different functional states of spleen cells from thymectomized and normal donors (*5*) and may provide a rational basis for understanding the immunoregulatory effects of thymic factors (*6*, *7*). In this context, the present results suggest that prostaglandins may play an important role in immunoregulation.

We have shown previously that a synthetic analog of PGE_2 mimics the effects of thymosin in inducing theta antigen in spleen cells (*1*) and the appearance of thymic-like activity in the serum (*2*) of thymectomized mice. Since the effects of thymosin were abrogated by indomethacin, we concluded that PGE might be the mediator of thymic factor for theta antigen expression. As shown in Fig. 3, the appearance of theta antigen, detected by the AZ inhibition assay, parallels the release of PGE_2.

The data reported here support the hypothesis that thymic factors act by stimulating PGE_2 production which appears to be responsible for at least some of the effects ascribed to thymic preparations—that is, the maturation of T lymphocytes (*7*). Also supporting this hypothesis are the data of Homo *et al.* (*8*) demonstrating increased amounts of prostaglandins in thymic epithelial cultures and those of Tomar *et al.* (*9*) showing that thymocytes are very sensitive to PGE_2. Oppenheim *et al.* (*10*), among others, reported that prostaglandins may influence T lymphocyte differentiation and maturation. Leport *et al.* (*11*) and Favalli and colleagues (*12*) reported that an analog of PGE_2 restored the immune response in immunosuppressed mice in vivo. Finally, Mertin and Stackpoole (*13*) demonstrated that antiserum to PGE suppressed cell-mediated immunity. Together, these data support the role of PGE in the regulation of the immune response, and the results of the present study indicate that one of these regulatory actions include the mediation of the activities of thymosin.

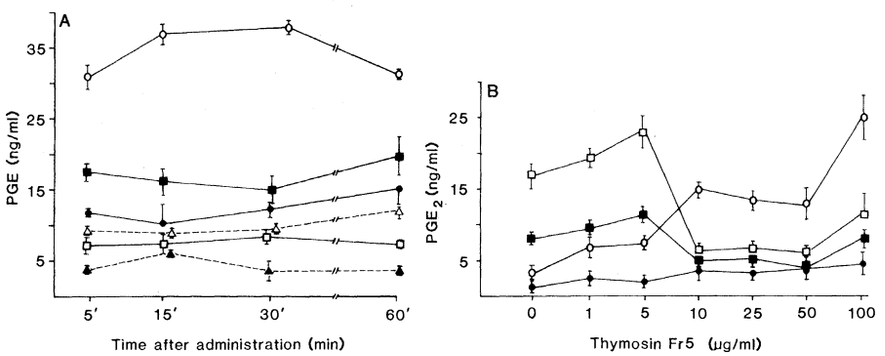

Fig. 1. (A) Release of PGE by spleen cells incubated with thymosin fraction 5 (Fr5). Four-week-old mice were thymectomized 10 days before the experiments. Spleen cells (3×10^6) from thymectomized mice were incubated with (○) Fr5 (100 μg/ml), (●) control diluent, or (△) with indomethacin (100 μg/ml) plus Fr5 in a total volume of 1.0 ml. After 5, 15, 30, and 60 minutes of incubation at 37°C in air with 5 percent CO_2, the supernatants were removed and the PGE concentrations were measured by means of a specific radioimmunoassay after extraction with a neutral organic solvent system and chromatography on silicic acid columns (*3*). Spleen cells (3×10^6) from intact mice were incubated under the same conditions (■, control diluent; □, Fr5; ▲, indomethacin plus Fr5) and the supernatants were harvested and analyzed for PGE concentrations. The PGE produced by spleen cells treated with indomethacin alone ranged from 7 to 9 ng/ml for those from intact mice and 3 to 6 ng/ml for similar cells from thymectomized mice. Data are expressed as arithmetic means ± standard error (S.E.). (B) Release of PGE by lymphocytes incubated with Fr5. Spleen lymphocytes (3×10^6) from (○ and ●) thymectomized and (□ and ■) intact mice were separated on Lymphoprep (Nyegaard, Oslo, Norway) and incubated with (○ and □) Fr5 (1, 5, 10, 25, 50, or 100 μg/ml) or with (● and ■) indomethacin (100 μg/ml) plus the same concentrations of Fr5 in a total volume of 1.0 ml. After 15 minutes the supernatants were removed and analyzed for PGE_2 concentrations by using a specific radioimmunoassay (*3*). The point indicated with 0 indicates no treatment with Fr5. Data are expressed as the arithmetic mean ± standard error.

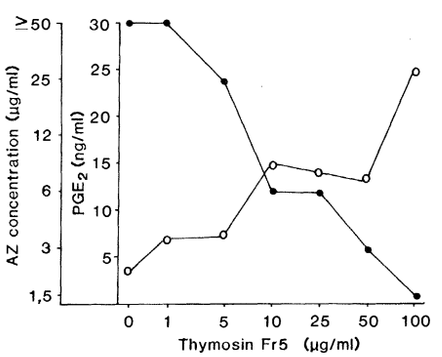

Fig. 2. Dose-response curves of lymphocytes from thymectomized mice stimulated with various amounts of Fr5 and tested in two parallel experiments to evaluate the appearance of (●) sensitivity to azathioprine (AZ) and (○) release of PGE. The appearance of AZ sensitivity, indicating the presence of theta antigen, was determined by the rosette inhibition test of Dardenne and Bach (*14*). Briefly, 3×10^6 spleen lymphocytes from thymectomized mice were incubated with increasing concentrations of AZ. After 90 minutes, 12×10^6 sheep red blood cells were added and the cell suspensions were centrifuged, the cells were gently resuspended, and the rosettes were read on a hemocytometer. The minimum concentration of AZ that reduced the number of rosettes in a given field by more than 50 percent compared to the control was taken as the active concentration. The AZ active concentration of normal spleen lymphocytes was 1 μg/ml. The point indicated with 0 indicates no treatment with Fr5.

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15. This work was supported by the grant 80.00450.04 from the Consiglio Nazionale delle Ricerche.

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13 December 1982; revised 10 March 1983