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Stimulation of human peripheral blood lymphocytes by Tamm–Horsfall urinary glycoprotein

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Summary. Tamm–Horsfall urinary glycoprotein (THP), prepared by salt precipitation of pooled urine from normal individuals, stimulated purified human peripheral blood lymphocytes (PBL) to undergo blastoid transformation. The response was measured by tritiated thymidine uptake into DNA after 6 days in culture. Several batches of THP stimulated, in varying degrees, all samples of PBL tested and the response approached that seen with the mitogens phytohaemagglutinin (PHA), Concanavalin A (Con A) and pokeweed mitogen (PWM) after 4 days in culture. The response usually exceeded that seen after 6 days with tuberculin purified protein derivative (PPD) in Mantoux positive lymphocyte donors.

INTRODUCTION

Tamm–Horsfall urinary glycoprotein (THP) was shown to be a potent inhibitor of myxovirus induced haemagglutination by its discoverers (Tamm & Horsfall, 1950), and is produced in the kidney tubules (Keutel, 1965; McKenzie & McQueen, 1969; Schenk, Schwartz & Lewis, 1971).

The function of this glycoprotein remains obscure, although pathologically it has been implicated in renal disease as a significant component of urinary casts (McQueen, 1962; Fletcher, McLaughlin, Ratcliffe & Woods, 1970). More recently, cell-mediated immunity and lymphocyte cytotoxicity against it have been reported in active chronic hepatitis and primary biliary cirrhosis associated with renal tubular acidosis (Tsantoulas, McFarlane, Portmann, Eddleston & Williams, 1974; Cochrane, Tsantoulas, Moussouros, McFarlane, Eddleston & Williams, 1976), and circulating THP antibodies have been found in the sera of patients with acute pyelonephritis (Hanson, Fasth & Jodal, 1976).

We report here the ability of THP to stimulate normal human blood lymphocytes to undergo *in vitro* blastoid transformation which has been measured by tritiated thymidine uptake by the DNA of the proliferating cells. This immunological property of THP has not been reported previously.

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MATERIALS AND METHODS

Separation of THP from urine

Urine was collected from both male and female laboratory workers and pooled. Batch volumes of between 2–5 litres were made 0·58 M with NaCl and stirred for 30 min, centrifuged at 1750 *g* for 10 min and the supernatant discarded. The precipitate was dissolved in alkaline distilled water (pH 9 with NaOH) and again centrifuged to remove any

insoluble material. The supernatant was then made 0·58 M with NaCl, centrifuged, and the precipitate washed twice with 0·58 M NaCl. This precipitate was resuspended in alkaline distilled water and left stirring overnight at 4°, again centrifuged, and any slight precipitate discarded. The slightly cloudy solution was dialysed extensively against distilled water at 4°, lyophilized and stored desiccated at −20° until used.

Separation of PBL from blood

Blood was collected in 2·7% EDTA, diluted 1 : 2 with Dulbecco's phosphate buffered saline (PBS) and layered over Ficoll-Hypaque (Böyum, 1968). This preparation consisted of >98% lymphocytes, the trypan blue exclusion test showing >98% viable cells.

Lymphocyte transformation conditions

Lymphocyte microcultures were carried out by a method modified from Penhale, Farmer, MacCuish & Irvine (1974). After purification on Ficoll-Hypaque, the lymphocytes were washed twice in Dulbecco's PBS and once in Eagle's minimal essential medium (EMEM). They were then resuspended to 1×10^6 lymphocytes per ml in EMEM containing 20 mM glutamine, 50 I.U. of penicillin and 50 μg streptomycin per ml. The medium was supplemented with 10% foetal calf serum (FCS) and buffered with 10 mM sodium bicarbonate and 12·5 mM HEPES. Triplicate 0·2 ml cultures were incubated in flat-bottomed microtitre plates (Cooke Microtitre), at 37° for 96 h (mitogens) or 144 h (THP and PPD), in humid air with 5% CO_2. Four hours before harvesting sample viabilities were measured, 0·4 μCi in 5 μl of 6-^{3}H thymidine (specific activity: 5 Ci/mM, The Radiochemical Centre, Amersham, Bucks.) was added to each well and the trays placed in a microshaker (Cooke Microtitre) for 10 s. The cultures were extracted on to fibreglass discs using a Skatron Multiple Cell-culture Harvester (Skatron AS, Lierbyen, Norway) and radioactivity measured in a Packard Liquid Scintillation Counter (Packard Tricarb). The results are mean values of triplicate wells.

Preparation of mitogens, THP and PPD for culture

PHA. (M form) (GIBCO Batch No. R.369002). PHA was diluted in EMEM and used in concentrations 5, 10, 20 and 40 μl/ml of culture.

PWM. (GIBCO Batch No. C.162206). This was diluted in EMEM and used at 0·25, 0·5, 1·0, 2·5 and 5·0 μg/ml of culture.

Con A. (Pharmacia, Batch No. 7001). Con A was prepared in EMEM and used at 1·0, 2·5 and 5·0 μg/ml of culture.

THP. THP was dissolved in EMEM without FCS at 2 mg/ml, filter sterilized through a 0·20 μ pore size filter (Gelman, U.S.A., G.A.-8) and FCS added to 10% by volume. This stock solution was further diluted with EMEM and used at 50, 100, 150, 200, 250, 300, 400 and 500 μg/ml of culture.

PPD. (Commonwealth Serum Laboratory, Australia, 2 mg/ml). This was dialysed extensively against distilled water to remove the preservative, lyophilized and stored at −20°. It was dissolved and filter sterilized in EMEM as above before being used at concentrations of 0·25, 0·5, 1·0, 5·0 and 10·0 μg/ml of culture.

Skin testing for PPD sensitivity

Tuberculin PPD (0·1 ml) (100 U) was injected intradermally into the forearm of laboratory staff and the induration measured after 48 h.

RESULTS

Five batches of THP separated from the pooled samples of urine stimulated human purified PBL in culture. In all, twenty-six samples of PBL from eighteen individuals, were tested against a range of concentrations and batches of THP. These experiments are summarized in Table 1 which shows the mean response and the range of THP concentrations at which the peak response was seen with individual batches. Results are c.p.m. from stimulated minus unstimulated cultures and are expressed as mean ± 1 s.d. of the individual samples at optimum THP concentration.

The range of THP concentrations at which the peak response was observed is seen to vary from 100–400 μg/ml of culture medium. This probably reflects changes in the activity of the different batches tested as well as variations in the responsiveness of individual lymphocyte populations. The size and range of the peak response across the spectrum of the five stimulatory batches is shown in

Table 1. Lymphocyte response to stimulatory batches of THP

Batch	Donors tested	% Donors positive	Mean c.p.m. ± s.d.	Optimum concentration range (μg/ml)
1	8	100	12,809 ± 10,479	100–200
2	5	100	15,496 ± 12,178	150–200
3	3	100	12,274 ± 9721	100–150
4	6	100	25,911 ± 7913	250–300
5	4	100	16,600 ± 12,829	300–400

Data obtained from stimulated minus unstimulated triplicate cultures of the donors' lymphocytes at optimum THP concentrations.

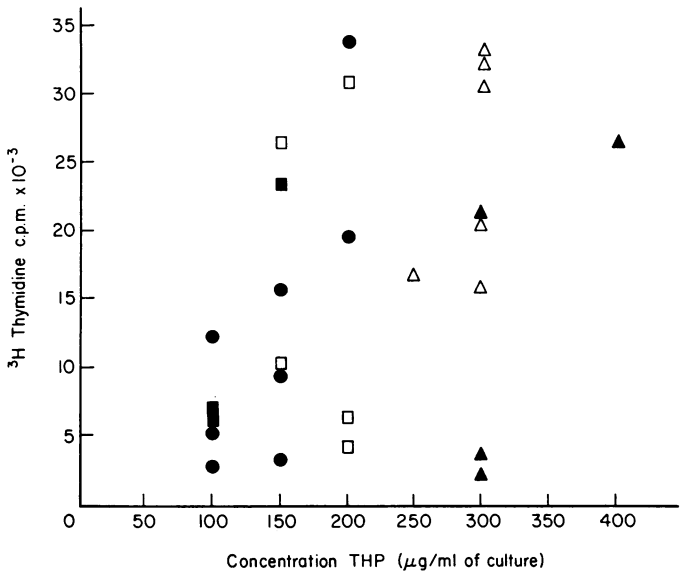

Figure 1. Range of lymphocyte responses to THP at optimum concentration. Each lymphocyte sample was cultured over a range of THP up to 500 μg/ml. Each point is the mean response of triplicate cultures from individual lymphocyte donors at the THP concentration which produced the peak response. (●) Batch 1, (□) batch 2, (■) batch 3, (△) batch 4, (▲) batch 5.

Fig. 1. The cluster observed between 100 and 300 μg/ml suggests this is the optimum range for stimulation with THP.

Comparison with mitogens

Control cultures using commercial mitogens were harvested at 4 days and set up in parallel with 6 day cultures testing a range of THP concentrations. PHA was used on all occasions, with Con A and PWM also being used when sufficient cells were separated from the sample of blood taken. Cells were usually > 85% viable after 4 days and > 70% viable after 6 days.

Data from these experiments (Fig. 2), which include results from cultures using all five stimulatory batches, show a mean peak response for THP of 17,223 c.p.m. Stimulation indices (S.I.) calculated by

$$\frac{\text{stimulated c.p.m.} - \text{unstimulated c.p.m.}}{\text{unstimulated c.p.m.}}$$

showed a mean of 61 (range 6–183). Both this value and the mean peak response approached that found with mitogens (mean S.I. values PHA 256, Con A 162, PWM 91). From these results THP appears to be an effective agent in stimulating lymphocyte blastoid transformation.

Table 2. Response from six Mantoux positive donors to PHA at 4 days and PPD and THP at 6 days

Donor	PHA Stimulation (c.p.m. ± s.d.)	THP Stimulation (c.p.m. ± s.d.)	PPD Stimulation (c.p.m. ± s.d.)	PPD Skin test (mm induration)
1†	29,865 ± 2281	3292 ± 645	13,818 ± 565	22
2†	58,812 ± 3969	2845 ± 541	12,222 ± 2739	20
3‡	55,105 ± 2414	32,190 ± 5644	6107 ± 1889	19
4‡	51,841 ± 5655	12,299 ± 1879	7269 ± 3248	15
5	46,622 ± 2424	6338 ± 1610	1857 ± 424	12
6‡	60,007 ± 4826	31,388 ± 1547	1933 ± 1120	8
7*	51,380 ± 5996		38,837 ± 1064	

Results are mean ± s.d. of (stimulated − unstimulated) lymphocyte cultures at optimum concentrations. Skin testing was read 48 h after 100 U PPD in 0·1 ml was injected intradermally into the forearm of the donor.

* Data from former tuberculosis patient.
†, ‡ Same batches of THP.

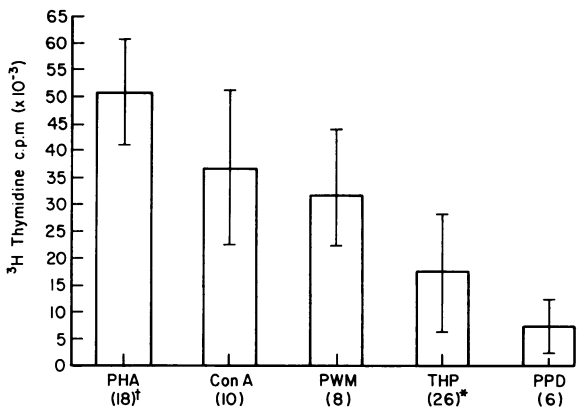

Figure 2. Comparative response from 18 donors tested against all stimulatory batches of THP. The height of each column represents the mean of the peak responses to the stimulating agent shown beneath it, bar is ± s.d. of the mean.

* This number exceeds that for PHA because some individuals were tested for more than one batch of THP.

† The number of individuals tested.

Comparison with PPD antigen

PBL from donors showing a positive skin test to PPD were cultured with a range of PHA, THP and PPD concentrations. Table 2 shows data from these individuals, the mean peak response to PPD of 7 201 c.p.m. being also shown graphically in Fig. 2. This value is seen to be about half that of the mean THP peak response. The mean S.I. value seen in these experiments was 36, similarly about half that of THP. Also shown in Table 2 are data from a former tuberculosis patient in clinical remission for 16 years. The response from this individual's PBL is of the same order as that of the mitogens when measured in c.p.m. alone although the S.I. of 66 seen in this case is almost the same as that generally observed with THP.

DISCUSSION

Tamm–Horsfall glycoprotein has been shown by this study to be a potent stimulator of *in vitro* blastogenesis in peripheral blood lymphocytes from normal individuals. It is capable of eliciting a response which approaches that of mitogens and is in excess of the usual PPD antigen response shown by the lymphocytes from our Mantoux positive individuals.

THP is a glycoprotein known to contain as much as 28% carbohydrate (Fletcher *et al.*, 1970), and in this respect resembles plant lectins with recognized mitogenic properties—PHA with 4·1–8·9% carbohydrate (Lis & Sharon, 1973) and some PWM fractions with up to 12·5% carbohydrate (Waxdal, 1974).

In its native form, THP is polymeric with a molecular weight of about 7×10^6 (Tamm, Bugher & Horsfall, 1955), and appears to be composed of subunits of about 100,000 mol. wt. (Fletcher *et al.*, 1970). Some PWM fractions are also polymeric in nature, a phenomenon suggested as necessary for some forms of lymphocyte activation by PWM (Waxdal, 1974) and also PHA (Yachnin, Allen,

Baron & Svenson, 1972; Miller, Hsu, Heinrikson & Yachnin, 1975).

Not all our batches of THP have proved to be capable of stimulating lymphocytes and we have been unable to distinguish between stimulatory and non-stimulatory batches of THP by a variety of immunochemical techniques. The lymphocyte activation may depend on the polymeric nature of the stimulating agent and an explanation for the electrophoretic similarity we have observed in stimulatory and non-stimulatory batches may lie in the particular polymeric form of the protein used during the *in vitro* test.

The question arises whether THP acts as a mitogen or as an antigen stimulating a specific clone of sensitized cells. As far as we are aware, none of the eighteen healthy donors of peripheral blood lymphocytes suffered from any form of renal disease and it seems unlikely that the response reported here indicates the presence of autoallergy to THP.

Cell-mediated activity against THP may be sought in patients with renal tubular damage and it is important to appreciate that THP may stimulate lymphocytes from apparently normal individuals.

The level of blastoid response, together with the observation that lymphocytes from all healthy donors tested responded when exposed to all stimulatory batches of THP, suggests a mitogenic rather than an antigenic stimulus. Further work is required to determine which lymphocyte population(s) take part in this reaction.

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