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THE PENICILLIN ANTIGENS: PRESENTATION IN THE LEUCOCYTE MIGRATION TEST

(A Study with Penicillin and Penicilloylated Proteins)

By

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The leucocyte migration test (LMT) was found to be a useful method for the *in vitro* study of cellular immunity to penicillin.

In a previous work, we have found (8) that LMT was positive in patients receiving penicillin who had no clinical symptoms of hypersensitivity. This was in agreement with the findings of Redmond & Levine (6); these authors demonstrated that the delayed skin reaction to an intradermal test with penicillin was often positive in non-allergic subjects.

However, a study by Ortiz-Ortiz et al. (4) led to a different result. LMT was only found to be positive in patients with an allergy to penicillin, but negative in non-allergic controls; these authors did not use penicillin as antigen, but penicilloylated gamma globulins.

As the presentation of the penicillin antigens could explain these discrepancies, we undertook a study in which the response of the LMT to penicillin was compared with the responses of the LMT to penicilloylated human gamma globulins and human serum albumin.

MATERIAL AND METHODS

Subjects

Forty-nine subjects were studied.

a) Thirty adults (24 males, 6 females; 25 to 65 years) without history of hypersensitivity to penicillin (subsequently quoted as "non-allergic"); 17 were tested more than 1 year after treatment with penicillin; 13 were studied 1 month after a well-tolerated therapy with penicillin (1 million units per day for 15 days), six of them for streptococcal skin infections and seven for serologic syphilis.

b) Nineteen well-documented hypersensitive subjects to penicillin (12 males, 7 females; 25 to 65 years) were tested 1 month after the end of the treatment which had induced the reaction. The clinical symptoms of hypersensitivity were: urticaria (eight cases), maculo-papular rashes (10 cases), anaphylactic shock (one case). These patients are subsequently quoted as "allergic".)

Leucocyte Migration Test (LMT)

The technique used was similar to Federlin's (1) which is a slight modification of the method described by Sjøberg & Bendixen (11). The criteria for a positive LMT have been reported previously (7, 8). Briefly, a 0.80 migration index was considered to be the highest value for a positive test. The significance of each inhibition was further assessed as follows: For each control migration series, the mean (MC) and the standard deviation (s.d.) were calculated; in each experiment reported as positive the mean of migration values with antigens (MA) was $MA \leq MC - 3 \text{ s.d.}$ (i.e. a significance at the 0.01 level). In a few cases a stimulation of the migration was observed; it was only noted as such and outlined when $MA \geq MC + 3 \text{ s.d.}$

Antigens

Benzyl penicillin (BP) (Specilline G Specia) was prepared extemporaneously and used at 1,000 and 10,000 IU/ml in 199 medium (Institut Pasteur) supplemented with 10 per cent horse serum (Institut Pasteur). The number of benzyl penicilloyl groups generated in this medium at 37° C was evaluated by means of the penamaldade assay according to Levine (3), at 3, 6 and 18 h. Cell-free supernatants from LMT chambers were also evaluated and gave similar results.

Benzyl penicilloylated human serum albumin (BPO HSA) was prepared according to Parker & Thiel (5) with an incubation time of 60 h. Human serum albumin was provided by CNTS (Paris).

There were 40 to 60 nmol BPO groups per nmol HSA as estimated by the penamaldade assay (3).

BPO HSA was used at 50, 100 and 200 µg/ml.

Benzyl penicilloylated human gamma globulin (BPO HGG) was prepared according to Haimovich et al. (2) with HGG provided by Diamant (Paris).

There were 21 nmol BPO groups per nmol HGG as estimated by the penamaldate assay (3).

BPO HGG was used at 50, 100 and 200 $\mu\text{g/ml}$.

In each case, the LMT responses to BPO HSA or BPO HGG were compared with the control responses to unpenicilloylated HSA or HGG at 50, 100 and 200 $\mu\text{g/ml}$ prepared as previously described but without penicillin.

RESULTS

Responses of LMT to the Different Penicillin Antigens

When the LMT was found to be positive with BP it was positive in 90 per cent of the cases with BPO HSA antigens and in 53 per cent with BPO HGG antigens (Table 1). When the LMT was negative with BP it was negative in 69 per cent with BPO HGG (Tables 2 a and 2 b); with BPO HSA, LMT was negative in 90 per cent of the cases (Table 2 a) or in 50 per cent (Tables 2 a and 2 b); see Table 3 for the reasons for this distinction. Nevertheless, there were no significant differences (χ^2) between the results obtained with BP, BPO HSA and BPO HGG on the whole.

The number of BPO groups generated in the culture media by penicillin (BP) is compared in Fig. 1 with the number of BPO groups supplied by the penicilloylated proteins. It can be seen that during the first 3 h, a concentration of 10,000 IU/ml of BP generated at least as many BPO groups as those carried by BPO HSA at 200, 100 and 50 $\mu\text{g/ml}$ and BPO HGG 200 $\mu\text{g/ml}$. No significant differences were found in the LMT responses between these five antigens which had supplied more than 25 nmol of BPO groups per ml at the third hour (Fig. 2).

A concentration of 1000 IU/ml of BP generated fewer BPO groups; the number of these were very similar to the number of BPO groups carried by BPO HGG antigens at a concentration of 50 and 100 $\mu\text{g/ml}$ (Fig. 1). The percentage of positive LMT (Fig. 2) was significantly lower ($P < 0.001$; χ^2) with these three antigens as compared with the other five.

TABLE 1

Positive LMT with Penicillin (BP) 10,000 IU/ml in a Comparison with the LMT Response to Benzyl-Penicilloylated Human Serum Albumin (BPO HSA) and to Benzyl Penicilloylated human Gamma Globulins (BPO HGG).

	BP	BPO HSA	BPO HGG
Non-allergic	0.70	0.65	ND
	0.52	0.63	ND
Non-treated	0.44	0.83 *	ND
	0.76	0.76	ND
	0.78	0.72	ND
	0.65	0.67	ND
	0.59	0.82 *	ND
	0.68	0.58	0.74
	0.70	1.64 *	ND
	1.55	1.23	1.39
Non-allergic	0.64	0.60	0.74
	0.50	0.52	0.57
Treated	0.73	0.53	0.71
	0.69	0.45	0.82 *
	0.66	0.74	0.87 *
Allergic	0.71	0.65	0.84 *
	0.77	0.70	0.59
	0.48	0.61	0.86 *
	0.58	0.66	ND
	0.70	0.61	ND
	0.46	0.57	0.79
	0.59	0.69	0.67
	1.26	0.50 *	0.81 *
Total	23	23	13
Inhibited	21	19	7
Stimulated	2	2	1

Numbers represent the more significant index reduction (or increase) obtained with the three concentrations of the penicilloylated proteins (i.e. most often 200 $\mu\text{g/ml}$ for BPO HGG; 100 or 200 $\mu\text{g/ml}$ for BPO HSA).

* Shows the differences between the responses to BP and BPO HSA or BPO HGG.

ND = not determined.

Responses to the Different Antigens According to the Clinical State

In the group of subjects *without* hypersensitivity to penicillin (Table 3) LMT was positive in 46.6 per cent of the cases with BP, in 28.5 per cent with BPO HGG antigens and in 48 per cent with BPO HSA antigens. In the group of subjects *with* hypersensitivity to penicillin there were no significant modifications in the percentage of positive LMT with BP or BPO HGG as compared with the non-allergic group. A significant increase was found, however, with BPO HSA antigens, from 48 per cent to 94 per cent ($P < 0.001$; χ^2).

There was no significant correlation between the clinical type of hypersensitivity to penicillin and the LMT responses to the antigens used in this study.

TABLE 2 (a)

*Negative LMT with Penicillin (BP) 10,000 IU/ml Compared with: (a) the LMT response to BPO HSA and to BPO HGG in Non-Allergic Subjects**; and (b) the LMT Response to BPO HSA and BPO HGG in Allergic Subjects†.*

	BP	BPO HSA	BPO HGG
Non-allergic	0.94	0.88	ND
non-treated	0.81	ND	1.05
	0.98	ND	1.02
	0.92	ND	1.07
	0.88	1.04	1.15
	0.81	1.09	1.04
	0.86	ND	0.87
Non-allergic	1.06	1.02	0.90
treated	1.07	ND	1.33 *
	1.17	0.83	1.49 *
	1.07	0.87	1.11
	1.19	1.11	1.20
	0.82	0.84	0.68 *
	1.13	0.67 *	0.75 *
	1.02	0.91	1.00
Total	15	10	14
Inhibited	0	1	2
Stimulated	0	0	2

TABLE 2 (b)

	BP	BPO HSA	BPO HGG
Allergic patients	0.85	ND	1.18
	0.98	0.68 *	1.22 *
	1.00	0.52 *	0.89
	0.95	0.57 *	0.76 *
	1.10	0.84	0.62 *
	1.01	0.47 *	0.88
	0.92	0.66 *	0.89
	0.98	0.62 *	ND
	0.92	0.72 *	ND
	0.89	0.65 *	0.97
	1.09	0.78 *	1.05
Total	11	10	9
Inhibited	0	9	2
Stimulated	0	0	1

(see Table 1 for abbreviations and details).

** The three antigens gave similar results.

† BPO HGG gave similar responses to BP; BPO HSA always induced a significant migration index reduction, except in one case.

TABLE 3

LMT Responses to Benzyl Penicillin (BP), Benzyl Penicilloylated Human Serum Albumin (BPO HSA) and Benzyl Penicilloylated Human Gamma Globulin (BPO HGG).

	Non-allergic *			Allergic		
	Nb	% Inhibited	% Stimulated	Nb	% Inhibited	% Stimulated
BP	30	46.6	3.3	19	36.8	5.2
BPO HSA	25	48	8	18	94	0
BPO HGG	21	28.5	14.2	15	33.3	6.6

The percentages of positive LMT in the non-allergic groups tested with BP or BPO HGG are similar to the percentages in the allergic group. The percentage of positive LMT with BPO HSA was significantly higher in the allergic group than in the non-allergic groups ($P < 0.001$).

* Treated and non-treated.

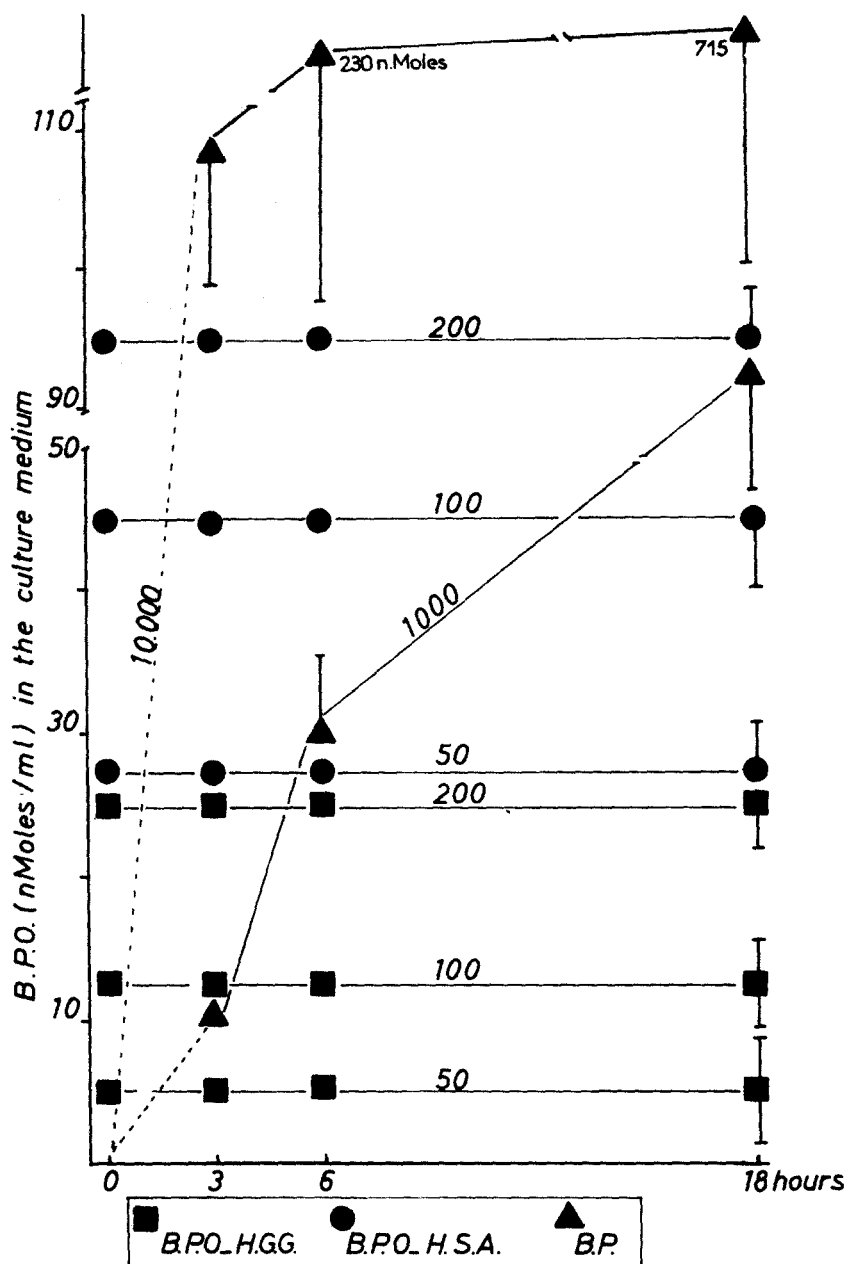


Fig. 1.

Benzyl penicilloyl groups (BPO) generated by benzyl penicillin (BP) 1,000 and 10,000 IU/ml in culture medium at 3, 6 and 18 h; the number of BPO groups generated is time- and dose-dependent. The number of BPO groups generated by BP is compared with the number of BPO groups supplied by the penicilloylated proteins (BPO HSA and BPO HGG, 50, 100 and 200 µg/ml). It can be seen that at the third hour, the number of BPO groups generated by BP 1000 and supplied by BPO HGG 50 and 100 µg/ml is lower than the BPO groups from the five other antigen presentations (see Fig. 2).

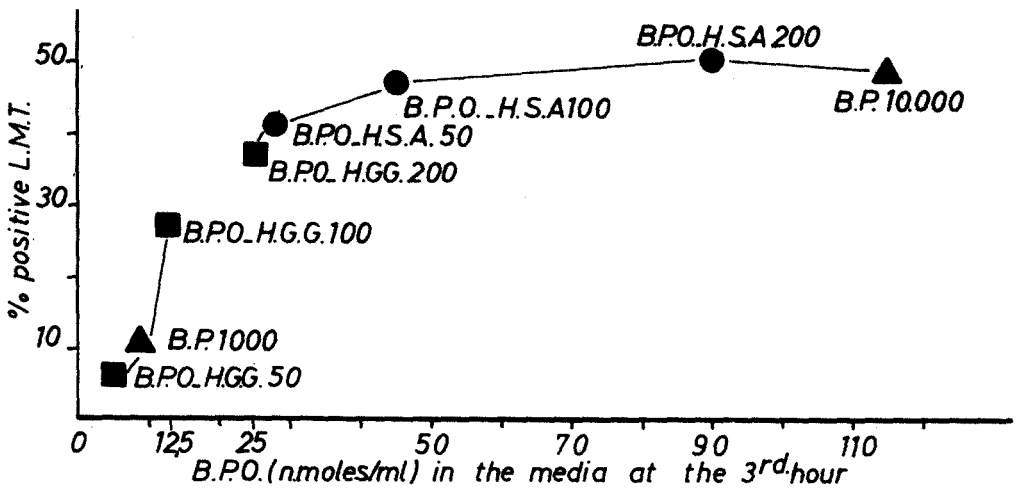


Fig. 2.

After 3 h the percentages of positive LMT were significantly higher ($P < 0.001$; χ^2) when the antigens supplied 25 nmol/ml (or more) of BPO, than when they supplied less than 12.5 nmol (For abbreviation see Fig. 1).

DISCUSSION

Using benzyl penicillin as antigen, we have previously found that the leucocyte migration test was a useful and sensitive method for the *in vitro* study of cellular immunity to penicillin (8). When BP is added to a culture medium, several antigenic determinants can be generated (9); these determinants can bind to several carriers, first of all proteins and cell membranes (10).

This work was undertaken to evaluate the role of the major antigenic determinant, the benzyl penicilloyl group (BPO); we also investigated the effectiveness of two human protein carriers, human gamma globulin and human serum albumin, which were found to be appropriate in the lymphocyte transformation test (12). The results were compared with those obtained with BP in the same patients.

When LMT was positive with BP (Table 1) it was most often positive with benzyl penicilloylated human serum albumin. With benzyl penicilloylated human gamma globulins the results were not so closely correlated; this fact probably reflects differences in the number of BPO groups (see later). However, there were no significant differences between the

responses of LMT to BP and to BPO HSA or BPO HGG. This finding supports the fact that mainly BPO specificity is involved, instead of other antigenic determinants, when BP is used as antigen.

The number of BPO groups present in the culture media at the third hour was found to be an important factor; indeed LMT was significantly less responsive when this number was low (below 12 nmol/ml). Antigens supplying more than 25 nmol/ml of BPO groups were found to induce better responses (Fig. 2). This finding calls for two comments:

1) The elicitation of the reaction by the antigens took place during the first three hours: as seen in Fig. 1 BP 1000 IU/ml generated as many BPO groups at the 6th h as BPO HSA 50 μ g/ml and BPO HGG 200 μ g/ml. However, the LMT was significantly less responsive with BP 1000 IU. Thus it can be thought that by the 6th hour, the elicitation was accomplished. This is in accordance with the chronology of lymphokine production.

2) When penicillin antigens are used in the LMT system, care must be taken to provide enough antigen during the first three hours. In this respect it is better to use penicilloylated proteins with a sufficient number of bound BPO groups instead of BP as such. We have demonstrated in this study that BP most probably acts through the formation of BPO groups. Penicilloylated proteins supply BPO groups immediately, whereas with BP, the BPO formation is time- and dose-dependent (Fig. 1).

When the results of LMT performed with different penicillin antigens were correlated with the clinical state (i.e. hypersensitivity to penicillin or not) two facts appeared:

1) LMT was positive with BPO HGG in subjects without hypersensitivity to penicillin (Table 3). This challenges the findings of Ortiz-Ortiz et al. (4). In their study only patients allergic to penicillin were found to respond to BPO HGG antigen. As the technique and the antigen were very similar in both studies the discrepancy is more likely due to differences in the choice of subjects.

2) LMT was significantly more often positive with BPO HSA in subjects who were allergic to penicillin than in those who were non-allergic (Fig. 3). As no difference of this kind was found with BP and BPO HGG, this finding is difficult to explain at present. BPO HSA was strongly penicilloylated and this can partially explain the results; work is in progress to evaluate other possibilities.

SUMMARY

The response of the leucocyte migration test (LMT) to benzyl penicillin (BP) was compared with the response of LMT to benzyl penicilloylated human gamma globulins (BPO HGG) and to benzyl penicilloylated human serum albumin (BPO HSA).

Patients with and without hypersensitivity to penicillins were investigated. No statistical differences were found between the response to BP and to BPO HGG or BPO HSA; this finding supports the fact that the BPO groups generated by BP are mainly implicated in the triggering of the LMT when BP is used as antigen. A strongly penicilloylated HSA appeared to be the most effective mode of the antigen presentation for the detection of BPO-specific cellular immunity in patients with and without clinical symptoms of hypersensitivity to the penicillins.

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