

Penicillin-allergic patients react to penicillin-modified "self"

S. J. D. BELL & W. J. PICHLER

Institut für klinische Immunologie, Inselspital Bern, Schweiz

Penicillin G (PNG) has been demonstrated to elicit T-cell responsiveness *in vitro* in allergic patients by means of a lymphocyte transformation test (LTT). As it was not clear how, or in what form, the stimulatory PNG determinants were inducing cellular proliferation, we compared the immune response elicited by different PNG preparations. Peripheral blood lymphocytes (PBL) of patients with proven PNG allergy were isolated, and proliferative responsiveness to soluble PNG alone, soluble PNG-protein conjugates (BPO-HSA, BPO-PL, BPO-HEX), and non-reactive penicilloate salts, was evaluated. An autologous mixed lymphocyte culture (MLC) using penicilloylated stimulator cells, was used to test responsiveness to membrane-bound PNG. We found that the addition of either 1000 µg/ml of potentially-reactive PNG to cell cultures, or of penicilloylated autologous cells was stimulatory, whereas non-reactive PNG salt, and soluble PNG conjugates were not stimulatory. Considering current and earlier findings, it appears that T cell immunity in these patients is directed towards PNG-modified "self", as PNG-modified autologous cells are potent stimulators in PNG-allergic individuals.

Key words: autologous mixed lymphocyte culture; lymphocyte transformation; penicillin.

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Allergic reactions to drugs occur by various immunological mechanisms (1, 8, 9). Most drugs, by virtue of their small size, are haptens, and are thus classified as incomplete antigens which are unable to elicit immune responses unless they become complete antigens (3, 13).

Certain drugs, which are reactive in their native state, or which have the potential to form reactive metabolites, can form complete antigens with relative ease (7). Complete antigens normally comprise a hapten-carrier complex, and as such they may now have the potential to elicit an immune response. The immune reactivity to an antigen is determined by the manner in which it is presented for recognition by the immune system. Reactive drugs can be presented on cell membranes, or alternatively, they may be presented after binding to soluble circulating proteins (12, 14). In the latter case, modified protein is taken up, and mainly a humoral immune

response to both the hapten and sometimes the carrier will occur.

Modified cell membranes may also elicit an immune response, which might be humoral or cell-mediated, or both (2). Penicillin is an example of a reactive drug that is capable of forming a covalent bond with amino or hydroxyl groups (6, 15). This reaction can give rise to both soluble and cell membrane-bound penicillin conjugates.

In this study we set out to determine whether the T cell proliferative response was directed against soluble or membrane-bound Penicillin G (PNG) conjugates.

Here, however, instead of analysing the reactive T cell phenotype, as we had previously done (10), we compared the immune response to different forms of penicillin antigen to see whether penicillin alone, soluble carrier conjugates of penicillin, non-reactive penicillin metabolites, and cell membrane-bound penicillin on intact cells, could stimulate T cells *in vitro*.

MATERIAL AND METHODS

Donors

The patients were recruited from our out-patient clinic on the basis of a history of penicillin allergy and as previous investigations had revealed a positive lymphocyte transformation test. The last allergic event had occurred at least 6 months ago, and in most instances more than 2 years before. Each time we performed a series of assays using PBL from an allergic patient, we carried out the same assays in parallel on a control donor with no history of allergy to drugs. Control subjects may or may not have previously been treated with penicillin.

Lymphocyte transformation test

The stimulatory effect of PNG alone, on PBL of both allergic and non-allergic donors, was first determined by measuring the uptake of ³H-thymidine by proliferating cells (16, 17).

Using the same protocol, T cell reactivity to soluble drug-carrier conjugates, (namely BPO-HSA, BPO-PL and BPO-HEX), as well as non-amino-reactive drug salt metabolites (α -ethyl-benzyl penicilloate and α -methyl-benzyl penicilloate) was tested. Here we used T cells of penicillin-allergic patients whose cells showed high Stimulation Index (SI) after challenge with PNG in the LTT. Drug-carrier conjugates used here, were prepared by the method described by Schneider et al. (11).

Autologous mixed lymphocyte culture (MLC)

The potential of penicilloylated autologous cells to stimulate an autologous MLC was tested. Purified PBL were divided into two fractions. After the addition of 20% v/v autologous plasma, untreated cells were kept separate to later serve as the responders (R). The second fraction was used to prepare penicilloylated autologous stimulators (S), to be used in autologous MLC. After washing the purified PBL, they were resuspended in Hepes-buffered (pH 7.4) RPMI 1640, without any additional addi-

Table 1
Comparison of LTT with autologous MLC SI results obtained after PNG stimulation of allergic patient PBL

Patients	Sex	Type of allergic reaction	LTT	Autologous MLC
I.S.	F, 1943	U,E	8.9	3.8
W.B.	M, 1945	E	3.8	1.5
M.B.	F, 1951	E	21.5	26.0
U.B.	M, 1948	AS	10.9	6.2
H.Z.	F, 1935	E,U	8.4	4.4
M.S.	F, 1926	AS,E	16.0	3.2
Mean SI:			11.5	7.5
Controls (6):				
Mean SI:			1.3	1.0
			(range: 1.0-2.1)	(range: 1.0-1.4)

Allergic reactions: E = exanthema; U = urticaria; A = angioedema; AS = anaphylactic shock; IN = interstitial nephritis.

Controls for the LTT were the cpm of each patient's cells in the absence of PNG (450-1450 cpm). Controls for the autologous MLC to penicilloylated cells, were determined as the autologous MLC without penicilloylated cells (260-1280 cpm). Non-allergic individuals did not show an enhanced proliferative response to penicilloylated autologous cells.

tives. 1000 μ g of PNG was added per ml of cell suspension (2×10^6 cells/ml), and incubated at 37°C for 16 h. S cells were then rendered metabolically inactive following 30 min treatment with 25 μ g/ml solution of Mitomycin-C (Syntex Pharma AG, Basel, Switzerland). Cells were then washed thoroughly in HBSS alone, thereby ensuring removal of soluble penicillin and penicillin-conjugates from the culture medium. Finally, to each well of the tissue culture plate which already contained 2×10^5 untreated R cells, we added 100 μ l of a 2×10^6 cell/ml penicilloylated S cell suspension. After 5-6 days incubation, cells were harvested and counted as with the MLC. Autologous MLC without PNG was used as control throughout.

RESULTS

All results are reported as a Stimulation Index (SI), which was derived as follows: cpm with penicillin/cpm without penicillin. Unstimulated

Table 2

Penicillin G, but not BPO-HSA, BPO-PL, BPO-HEX are potent stimulators of PNG-specific T cells

Patients	M.P.	H.J.	F.L.	U.B.	Z.H.	A.F.
Sex	M	F	F	M	F	F
Year of birth	1935	1940	1945	1948	1938	1922
Types of allergic reactions	E	U,E	U,A	AS	AS	IN
Control: (cpm × 10 ³)	1.3	0.4	0.7	0.7	0.7	0.8
PNG 1000 µg/ml	24.5	13.3	11.4	35.7	47.8	4.8
BPO-HSA	3.3	2.9	3.9	0.9	0.8	-
BPO-PL	1.5	2.9	4.0	28.1	1.3	0.7
BPO ₂ -HEX	1.7	3.6	1.8	0.9	0.6	-
BPO-PL Skin Test	+	+	+	+	+	+

Allergic reactions: E = exanthema; U = urticaria; A = angioedema; AS = anaphylactic shock; IN = interstitial nephritis. BPO-HSA = benzylpenicilloyl-human serum albumin; BPO-PL = benzylpenicilloyl-poly-L-lysine; BPO-HEX = benzylpenicilloyl-diaminohexane.

Table 3

Penicillin G (PNG), but not its non-reactive metabolite benzyl-penicilloic-acid/salt is stimulatory for T cells

Patients		T.R.	I.S.	P.G.	K.B.
Sex		F	F	M	F
Year of birth		1925	1943	1950	1930
Types of allergic reaction		E,AS	U,E	AS	E,AS
Control (cpm × 10 ³)		0.4	1.0	1.4	0.4
PNG 1000 µg/ml		6.0	5.6	5.8	32.8
α-ethyl-benzyl penicilloate	100 µg/ml	1.7	0.7	0.9	1.5
	200 µg/ml	2.0	0.7	0.4	1.0
	500 µg/ml	1.1	1.0	0.5	1.2
α-methyl-benzyl penicilloate	100 µg/ml	1.4	1.6	0.3	1.9
	200 µg/ml	1.3	0.7	1.1	1.3
	500 µg/ml	1.0	0.8	1.0	1.9

Controls were the cpm of each patient's R cells alone.

cultures had 450 to 1450 cpm. A positive stimulation was considered if the SI was ≥ 2.0 (7). Comparison of the results obtained in the LTT with the autologous MLC are shown in Table 1. LTT results confirmed that cells of the penicillin-allergic patients responded strongly to stimulation with PNG. After 5–6 days continuous exposure to culture medium containing 1000 µg/ml of PNG, the mean SI value recorded was 11.5. This was significantly higher than the mean SI of 1.3 recorded in the non-allergic control group donors. The mean SI value obtained following stimulation by penicilloylated

autologous cells was 7.5, which was not statistically different to the mean value obtained using the LTT (Student's two-tailed t test). Results obtained using soluble drug-carrier conjugates (i.e. BPO-HSA, BPO-PL, BPO₂-HEX) to stimulate T cells, compared with the stimulation seen to penicillin alone, are shown in Table 2. Here it is clearly seen that addition of amino- or hydroxyl-reactive penicillin alone, was more stimulatory than the addition of pre-formed penicillin-carrier conjugates, and only one donor mounted a high proliferative response against the BPO-PL conjugates.

Results shown in Table 3 clearly indicate that penicillin-sensitive cells were not stimulated to respond after exposure to non-reactive penicilloyl salts, α -ethyl-benzyl penicilloate or α -methyl-benzyl penicilloate.

Penicillin-specific IgE RAST was performed on sera from allergic patients. An elevated titer was not usually seen (results not shown).

DISCUSSION

In this study we analysed the mechanisms involved in the immune responsiveness of allergic individuals to penicillin antigen. Our results show that neither a non-reactive penicillin-salt, nor preformed soluble penicillin-conjugates (BPO-HSA, -PL, -HEX), could consistently elicit a proliferative response. Strong proliferative responses were, however, seen to occur following the addition of either 1000 μ g/ml of potentially reactive PNG or penicilloylated cells to cell cultures.

These T cell stimulatory events are likely to occur by similar mechanisms. The addition of 1000 μ g/ml PNG to cell cultures, or the use of penicilloylated autologous cells to stimulate antigen-specific proliferation, elicited the same amounts of responsiveness. In both protocols, a 1000 μ g/ml concentration of PNG was initially added. Both in the washed and unwashed cultures, only about 0.1% of the added PNG remains bound to the cells after washing (7). We show here that this small quantity of PNG is able to stimulate proliferation of PNG-specific cells in culture. That the T cell response is mainly directed to modified cell membranes, is further supported by the previously mentioned findings of Claas et al. (5), de Weck et al. (7), and Koponen et al. (10). Claas et al. (5) reported that of the membrane protein structures to which haptenic penicillin is known to adhere, HLA antigens are known to be important. In view of this fact, it is possible that penicillin could elicit an immune response to altered HLA determinants. In our previous studies we used the lymphocyte transformation test (LTT) to demonstrate that penicillin could be used to stimulate responsiveness of sensitized T cells (10). We analysed the phenotype of the reactive cells and

found that following *in vitro* stimulation, inducer cells for T suppressor/cytotoxic cells bearing the CD 4⁺ Leu 8⁺ surface markers were preferentially stimulated. The same T cell subset was also stimulated if the T cell response was directed against virally infected cells. The response to soluble antigens, such as tetanus toxoid or PPD was, however, different, as the CD 4⁺ Leu 8⁻ cells were stimulated. This suggested that the manner in which antigenic presentation of penicillin on the cell membrane occurred was similar for viral antigen presentation in association with HLA class 1 determinants (4). Taking into account earlier findings, and those with the patients in this study, we may draw two main conclusions.

Firstly, to elicit a T cell response, PNG must be reactive, and have the potential to bind to and modify "self" cell surface protein. Secondly, the immune response is directed against modified-self cell membranes; and preformed soluble PNG-carrier conjugates are unable to stimulate an *in vitro* response, because T cells are primed to react to membrane-bound, but not soluble PNG-carrier conjugates.

Our patient U.B. was seen to respond to PNG alone, and to the soluble BPO-PL conjugate. One may speculate that T cell responses of different specificities were induced. Whether these various kinds of T cell responses are also decisive for a varying clinical picture, is not yet clear.

The finding described here, namely that a drug elicits an immune response after modifying cell surfaces, may also account for certain adverse reactions to other drugs. In sensitized patients or animal models, it should be possible to use the technique of modifying autologous cell membranes, with other haptenic drugs or antigens, to evaluate potential cellular immune responses to the drug.

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Address:

Stephen J. D. Bell

The Basel Institute for Immunology
Grenzacherstrasse 487
CH-4005 Basel
Switzerland

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