

An analysis of β -lactam-derived antigens on spleen cell and serum proteins by ELISA and Western blotting

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Penicillins and related β -lactam antibiotics are known to conjugate to proteins to generate potentially antigenic (haptenic) determinants. In the present study, we used a rabbit polyclonal antibody raised against benzylpenicillin (BP) to investigate the capacity of six penicillins and one cephalosporin to generate haptenic groups *in vitro* on cultured mouse spleen cells and on serum proteins in the culture medium. All of the drugs tested, namely, BP, amoxicillin (AMX), ampicillin (AMP), cephalothin (CEP), cloxacillin (CLX), flucloxacillin (FLX), and phenoxymethylpenicillin (PMP) generated antigens in a concentration-dependent manner on cell and serum proteins, which could be detected by ELISA, although antigens generated by BP, CEP, FLX, or PMP in either cell- or serum-conjugated form were more readily detected than those generated by AMX, AMP, or CLX. Western blot analysis revealed that BP-derived antigens were generated relatively slowly on cell proteins (maximum binding was not yet reached after 8 h), compared to serum proteins (maximum binding within 1 h). BP, CEP, and PMP all generated similar distinctive patterns of immunostaining of electrophoresed cell or serum proteins which did not reflect the relative abundance of different proteins as revealed by Coomassie brilliant blue staining. FLX, CLX, AMP, and AMX did not generate antigens that could be detected on Western blots. In conclusion, we have shown that various β -lactam antibiotics generate antigens on cell and serum proteins that can be detected and characterized immunochemically with polyclonal antiserum. Further application of these methods may offer potential for further identification of immunologically relevant cellular and serum antigens generated by these drugs.

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Penicillins are widely used antibiotic drugs that are generally not toxic but in certain cases can cause various allergic side-effects varying from mild skin rashes to, at worst, anaphylactic shock and death (5, 10, 18). These symptoms often resemble those seen in classical immediate or delayed hypersensitivity reactions, and may be associated with drug-specific IgE antibody (2, 3, 11) and/or drug-specific T cells (1, 6, 9, 13). To induce IgE-mediated or cell-mediated

immune responses, a drug must conjugate to self-proteins to generate an haptenic immunogen that after processing can be presented in association with MHC class II molecules to T cells (reviewed in references 4, 17). β -lactam antibiotics conjugate to proteins by amide linkage through the carbonyl group of the opened β -lactam ring (18). Studies with radio-labelled benzylpenicillin revealed that a small proportion of the drug becomes conjugated to plasma proteins in this way *in vitro* and *in vivo* (12). We reported also, by immunoassay, generation *in vitro* of penicillin-derived antigenic determinants on the surface of macrophages (16). However, very little qualitative information is available on the nature of plasma, tissue, or cellular proteins that are targets for penicillin binding.

Abbreviations. AMP, ampicillin; AMX, amoxicillin; BP, benzylpenicillin; BPO, benzylpenicilloyl; CEP, cephalothin; CLX, cloxacillin; cRPMI, complete RPMI-1640 medium containing 5% foetal calf serum; ELISA, enzyme-linked immunosorbent assay; FLX, flucloxacillin; HSA, human serum albumin; PBS, phosphate-buffered saline; PBS-T, PBS containing 0.05% Tween 20; PMP, phenoxymethylpenicillin; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

The aim of this study was to look qualitatively at the capacity of a range of penicillins and one cephalosporin to generate antigenic determinants on spleen cells and serum proteins. We employed an *in vitro* system, based on that of O'Donnell et al. (16), in which cells are cultured with a drug in serum-containing medium; conjugation of the drug to serum and cells is then examined by enzyme-linked immunosorbent assay (ELISA). To detect drug-derived antigen, we used a polyclonal rabbit antibody raised against benzylpenicillin but with broad specificity toward various penicillins and related antibiotics differing in side-chain structure. In addition, to investigate the molecular targets for penicillin conjugation reactions, we used the same polyclonal antibody to analyse patterns of binding to spleen cell and serum proteins by Western blotting. A similar approach has been used successfully to detect penicillin-binding proteins in certain bacterial strains (8). The measurement and characterization of penicillin binding to immune cells and serum proteins may provide a useful predictor of the potential of the drug to act as a T-cell-stimulating agent and may allow further understanding of penicillin-induced immunotoxicity.

Material and methods

Reagents

Benzylpenicillin (BP), cephalothin (CEP), human serum albumin (HSA, fraction V), Tween 20, and *o*-phenylenediamine were obtained from Sigma Chemical Co, Poole, UK. Amoxicillin trihydrate (AMX), ampicillin sodium (AMP, Doktacillin), cloxacillin sodium (CLX, Eckvacillin), flucloxacillin sodium monohydrate (FLX), and phenoxymethylpenicillin potassium (PMP) were kindly provided by Dr K. Cederbrandt (ASTRA, Sweden). RPMI-1640 medium and foetal calf serum were obtained from Gibco (Paisley, UK). Conjugates of benzylpenicilloyl-human serum albumin (BPO-HSA) (hapten density 7:1), AMX-HSA (6:1), and AMP-HSA (12:1) were synthesized as described previously (12, 20). Rabbit antiserum specific for protein-conjugated BP was raised by injection of BPO-keyhole limpet haemocyanin conjugate emulsified in Freund's complete adjuvant (12), and was adsorbed against HSA. Affinity-purified peroxidase-labelled goat antirabbit IgG was obtained from Tago Inc, Burlington, CA, USA.

ELISA for characterization of rabbit anti-BPO antiserum

Microtitre plates were coated with various penicillin-HSA conjugates (10 μ g/ml, 125 μ l/well) in 0.15 M

phosphate-buffered saline (PBS), pH 7.2, overnight at 4 °C. The following were then added (100 μ l/well) sequentially with washing in PBS-0.05% Tween-20 (PBS-T) between each step: the rabbit antiserum diluted threefold down columns in PBS-T (1 h); peroxidase-labelled goat antirabbit IgG (1:4000 dilution in PBS-T, 1 h); and freshly prepared substrate solution containing 4 mg of *o*-phenylenediamine and 10 μ l of H₂O₂ per 10 ml of citrate-phosphate buffer, pH 5.0. The reaction was terminated after 10 min by the addition of 25 μ l of 25% H₂SO₄ to each well. Absorbances were read on an automated plate reader (Dynatech MR600) at a wavelength of 450 nm with the reference wavelength set at 630 nm.

ELISA for detection of β -lactams conjugated to cells and serum protein

Erythrocyte-depleted spleen cells from female CD1 Swiss white mice were suspended in RPMI-1640 medium containing 5% FCS, 40 μ g/ml gentamicin and 2 mM L-glutamine (complete RPMI, cRPMI). The cells were divided into 1.0 ml volumes containing 5×10^6 nucleated cells and were incubated with varying concentrations of drug at 37 °C for 4 h. After incubation, the cells were pelleted by centrifugation for 5 min at 300 g, and the supernatant medium was retained. The cells were washed three times in cRPMI and finally resuspended at a cell concentration of 5×10^6 /ml and dispensed into 96-well flat-bottomed cell culture plates (Costar), 100 μ l/well. The cells were fixed to the plates with glutaraldehyde, as described previously (16). The retained culture supernatant medium was added to the wells of microtitre plates (final protein concentration 50 μ g/ml), which were then incubated overnight at 4 °C. Plates coated with cells or serum proteins were then washed, and bound penicillin was detected by ELISA as described above, using the rabbit anti-BPO antibody at 1:1000 and the secondary antibody at 1:4000.

Western blotting

Murine spleen cells were incubated with BP over the concentration range 125 μ g/ml to 10 000 μ g/ml overnight or were incubated with a single drug concentration for varying periods of time. In further experiments, cells were incubated with AMX, CEP, AMP, CLX, FLX, or PMP at a concentration of 1 mg/ml for 24 h. After the appropriate incubation, the cells were pelleted and the supernatant medium containing serum proteins was retained. The cell pellet was washed twice with cRPMI and twice with PBS by centrifugation. It was then resuspended in 100 μ l of PBS and an equal volume of gel loading buffer containing the reducing agent dithiothreitol (3 mg/ml)

(14). The supernatant medium was diluted 1:1 (v/v) with gel loading buffer, also containing dithiothreitol. Proteins extracted from untreated and drug-treated spleen cells and supernatant media from these experiments were separated by sodium dodecyl sulphate–10% polyacrylamide gel electrophoresis (SDS–PAGE) (14). “Rainbow” molecular mass markers, size range 14.3–200 kDa (Amersham) were run on every gel. The separated proteins were then transferred to nitrocellulose (Schleicher and Schuell) according to Towbin et al. (19), for 30 min at 200 mA, with a semidry blotter (Biometra). The blots were blocked in 1% bovine serum albumin in PBS for 1 h at room temperature, washed in 0.1% Tween 20 in Tris-buffered saline (TBS-T), and then incubated in rabbit anti-BPO antibody or normal control rabbit serum (1:500 in TBS-T) overnight at 4°C on an orbital shaker. After incubation, blots were washed with TBS-T and then incubated with horseradish peroxidase-conjugated antirabbit IgG for 2 h at room temperature. Blots were then washed in TBS-T with a final wash in TBS and developed in TBS containing 0.05% 4-chloronaphthol and 0.133% H_2O_2 . Duplicated gels of plasma and cell protein samples were stained overnight for total protein with Coomassie brilliant blue, destained, and dried.

Results

Specificity of the rabbit anti-BP antiserum for synthetic penicillin-HSA conjugates

The rabbit antiserum raised against BPO-keyhole limpet haemocyanin recognized strongly BPO-HSA, but relatively weakly AMX-HSA or AMP-HSA (Fig. 1), even though AMX and AMP were conjugated to HSA to similar or greater hapten densities than BP. On the basis of these results, in all subsequent ELISAs for detection of antigen, the antibody was used at a dilution of 1:1000.

Generation of drug-derived determinants on spleen cells and serum proteins

Each of the six penicillins and the one cephalosporin generated antigenic determinants on the surface of splenocytes (Fig. 2a). Antigen generation was dependent on the concentration of the antibiotic over the range 125 µg/ml to 10 000 µg/ml (Fig. 2a). When normal rabbit serum was used in place of the anti-BP antibody, no binding of rabbit IgG to penicillin-treated cells was seen (Fig. 2a), confirming the specificity of the ELISA for drug-derived BP-cross-reactive determinants. AMX, AMP, and CLX generated antigen that was only weakly detectable, while BP, FLX, PMP, and CEP generated detectable cellular antigen more readily (Fig. 2a). Interest-

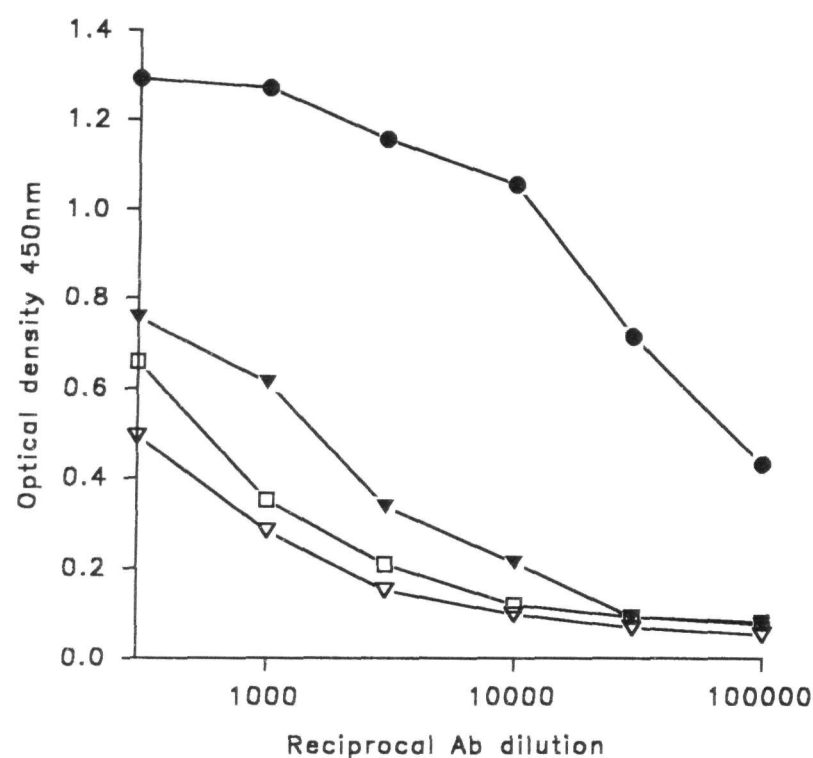


Fig. 1. Specificity of rabbit anti-BPO antiserum determined by ELISA. Wells were coated with synthetic conjugates BP-HSA (●), AX-HSA (▼), AMP-HSA (□), or unconjugated HSA (▽), and antiserum was diluted threefold down columns. Each point is mean of five wells. Similar results were seen in each of three experiments.

ingly, at the lower concentrations tested (125–500 µg/ml), the penicillin FLX showed the greatest detectable antigen generation of all the antibiotics tested in spite of the fact that the antiserum was raised against BP. It was also noteworthy that at the highest drug concentration tested (10 mg/ml) the cephalosporin CEP showed the greatest detectable generation of cell-surface antigen, with FLX giving a slightly weaker signal, while BP and PMP showed intermediate generation of detectable antigen (Fig. 2a).

As with the generation of drug-derived determinants on spleen cells, the generation of determinants on foetal calf serum proteins was concentration-dependent for each of the compounds tested (Fig. 2b). Again, no binding of normal rabbit IgG to BP-treated serum samples was detected, confirming the specificity of the assay for drug-derived BP-cross-reactive antigens (Fig. 2b). Generally, particularly at the highest concentration of compound used, the serum proteins gave a higher optical density reading than did the corresponding cell sample. This may reflect differences in the amount of protein bound to the microtitre plate rather than differences in the antigenicity of serum- or cell-associated determinants. The rank order of the various antibiotics in terms of their capacity to generate antigens on serum proteins was similar to that seen for spleen cell-surface antigen: for example, at 1 mg/ml of antibiotic, FLX and CEP produced the greatest detectable antigen, BP and PMP were intermediate, and AMX, AMP, and CLX were relatively weak. The rank order

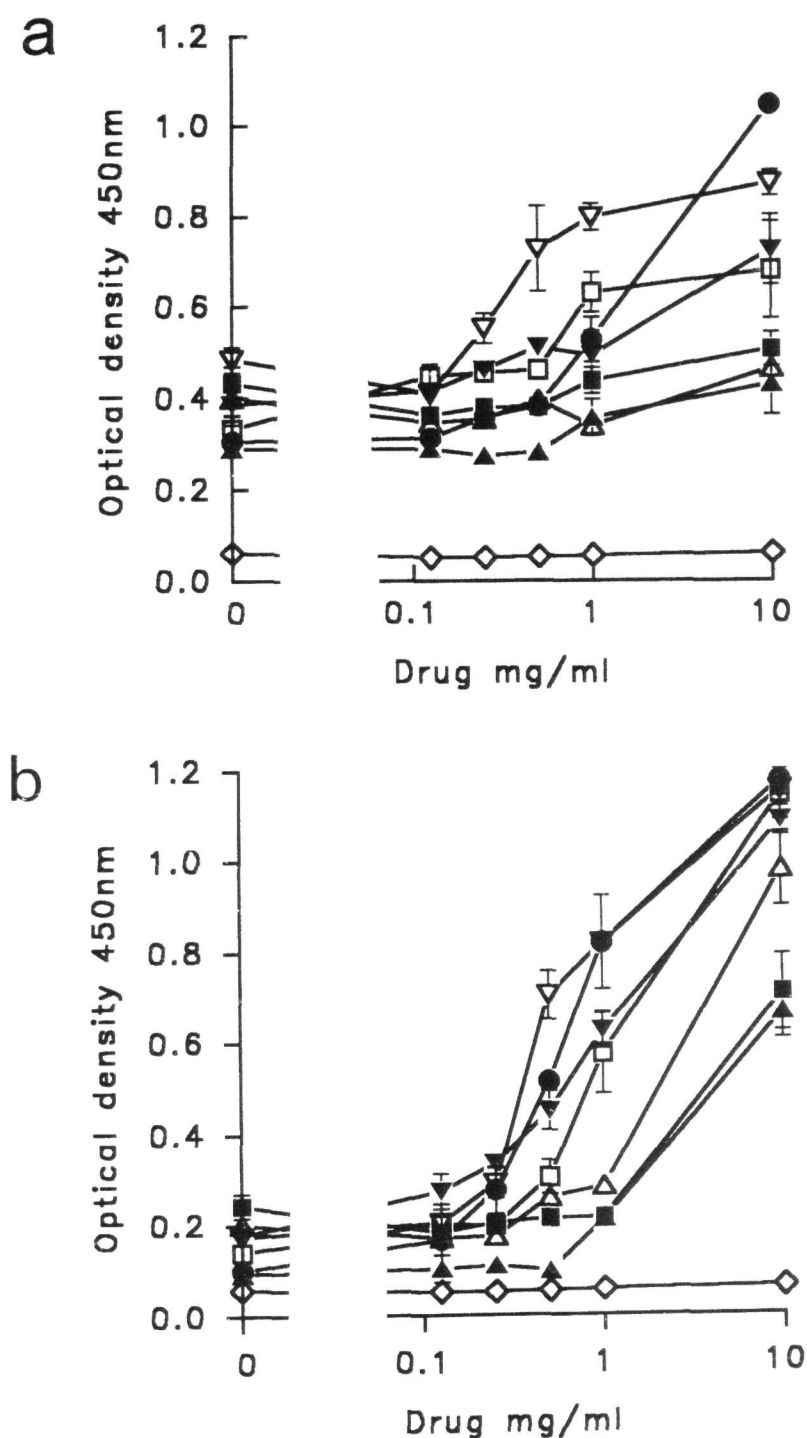


Fig. 2. Detection of drug-derived determinants on a) mouse spleen cells or b) foetal calf serum. Spleen cells were incubated in cRPMI with various concentrations of each drug for 4 h at 37°C, and cells and culture medium serum were then analysed by ELISA for drug-derived antigen. Each point is mean of five determinations, and SEM were typically less than 10% of mean values. $\triangle$ AMP, $\blacksquare$ AMX, $\blacktriangledown$ BP, $\bullet$ CEP, $\blacktriangle$ CLX, ∇ FLX, $\square$ PMP. $\diamond$ cells and serum treated with BP but normal rabbit serum in place of anti-serum.

was similar at the highest drug concentration: again FLX, CEP, BP, and PMP generated considerably more signal than AMX and CLX, while AMP was intermediate (Fig. 2b).

Western blot analysis of drug-derived cell and serum antigens

By Western blotting, we examined patterns of BP-haptenation of cell and serum proteins. In all experiments, control blots developed with normal rabbit serum in place of rabbit anti-BP antibody were nega-

tive, confirming the specificity of the assay for BP-cross-reactive antigens. In a first series of experiments, cultured spleen cells were treated with BP in the concentration range 10–10 000 μ g/ml for 4 h. BP-positive cell and serum bands first appeared in protein extracts from spleen cells cultured with 100 μ g/ml, and a progressive increase in the number and intensity of positive bands was seen as the BP concentration was increased progressively up to 10 000 μ g/ml (data not shown). In subsequent experiments, the cultured cells were treated with 1 mg/ml BP for 1, 4, 8, or 16 h (Fig. 3). Quite strikingly, haptenation of cell and serum proteins progressed at different rates. On cellular proteins, haptenated bands were generated relatively slowly: BP antigen was not seen until 4 h of incubation, and was considerably stronger at 18 than 8 h (Fig. 3a). In contrast, maximum BP haptenation of serum proteins was seen after only 1 h; no further increase in staining intensity was seen after this time (Fig. 3a). Coomassie brilliant blue staining of the electrophoresed cell and serum protein samples revealed identical patterns and intensity of protein bands, thus showing no differences in their protein content (Fig. 3b).

Cell and serum proteins showed distinct patterns of protein haptenation. Predominant BP-positive bands were seen on spleen cell proteins at positions corresponding to molecular masses of approximately 22–25 and 34–39 kDa, and multiple bands were seen above 50 kDa. Positive bands were seen on serum proteins at positions corresponding to molecular masses of approximately of 32–34 and 66 kDa (presumably albumin) and also either side of the albumin. By comparison of the Western blots shown in Fig. 3a and the Coomassie brilliant blue-stained electrophoresed proteins in Fig. 3b, it is clear that patterns of haptenation of either cell or serum proteins did not reflect the relative abundance of different proteins in the samples. For example, spleen cell proteins of molecular size below 21 kDa stained intensively with Coomassie brilliant blue but very weakly or not at all with the anti-BPO antibody. Similarly, an intensely Coomassie brilliant blue-stained band at 46 kDa did not stain with the antibody. Conversely, a number of large proteins (> 50 kDa) stained quite strongly with the antibody but weakly with Coomassie brilliant blue. With regard to the serum proteins, Coomassie brilliant blue revealed a predominant broad band (presumably albumin) that migrated with the 69-kDa marker, a second strong broad band just behind this, and a single but narrow strong band well ahead of the albumin (Fig. 3b). In contrast, the antibody stained a wide spectrum of serum proteins, many of which were not stained by the Coomassie brilliant blue and therefore presumably represent relatively minor protein constituents of serum.

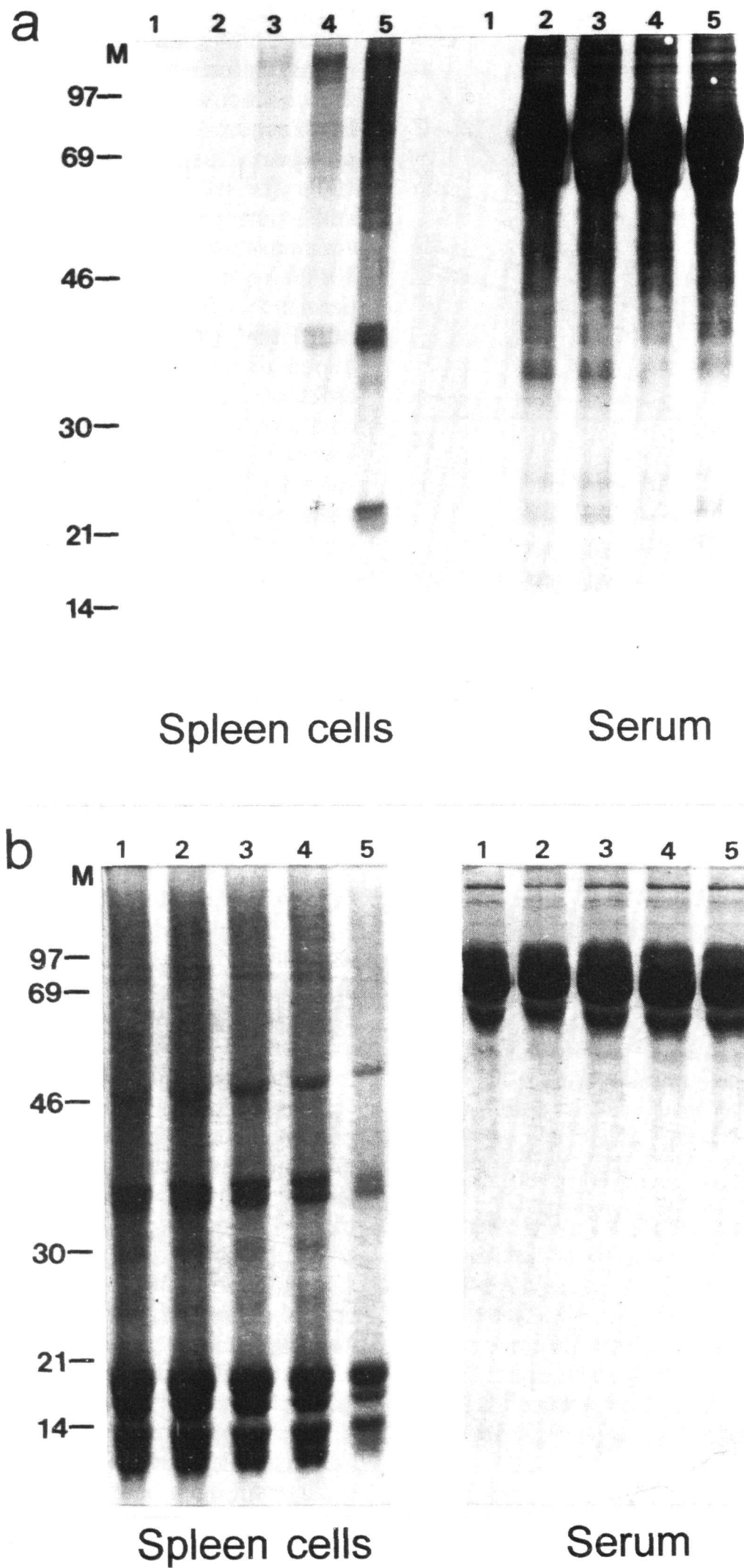


Fig. 3. a) Western blot analysis of kinetics of BP antigen generation on cell and serum proteins; b) Coomassie brilliant blue staining for total protein of same samples. Cells were incubated in cRPMI with 1 mg/ml BP for 1 h (lane 2), 4 h (lane 3), 8 h (lane 4), or 18 h (lane 5). Lane 1 shows untreated cells or serum. BP-derived antigen was detected with anti-BPO antibody at 1:1000 dilution. M = molecular mass markers.

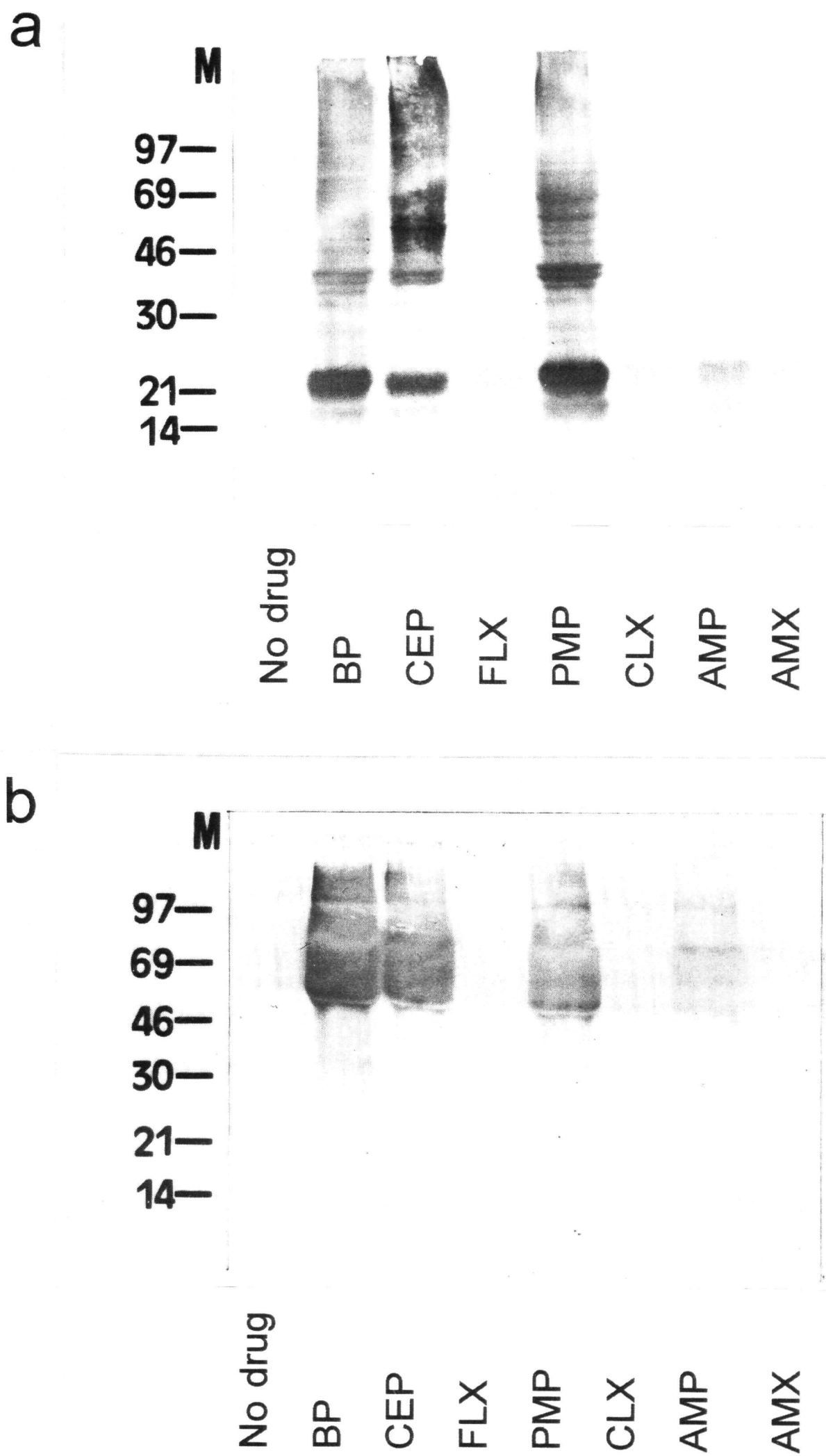


Fig. 4. Western blot analysis of total proteins extracted from (a) spleen cells or (b) serum after incubation for 24 h with 1 mg/ml of each of drugs as indicated. M = molecular mass markers.

In a further series of experiments, we compared patterns of antigen generation on spleen cell and serum proteins by the six penicillins and one cephalosporin that we had already studied by ELISA (see above). Cultured spleen cells were treated with the various antibiotics at 1 mg/ml for 24 h. Surprisingly, the drugs did not show the same rank order of intensity of antigen generation detected by Western blots as had been seen with ELISA. Western blots showed that, of the drugs tested, only BP, PMP, and CEP generated intense detectable positive bands on cell and serum proteins. The other four drugs hardly generated detectable positive bands, although AMP was a little stronger than CLX, FLX, and AMX (Fig. 4). BP, PMP, and CEP all generated a similar pattern of haptenated proteins on both cell (Fig. 4a) and serum (Fig. 4b) proteins. Coomassie brilliant blue staining of the same protein preparations revealed constant patterns and intensities of the various protein bands (not shown).

Discussion

We have shown that a range of six penicillins and one cephalosporin can generate antigenic determinants on cultured mouse spleen cells and culture medium serum proteins. By ELISA, the various β -lactams generated different levels of detectable antigen but showed a similar rank order of potency in terms of generation of antigen signal on both cellular and serum proteins. We are unable to say with certainty whether these differences between drugs reflect quantitative differences in binding or differing affinity of the antiserum for the different antibiotics. However, the rabbit antiserum used in these immunochemical investigations was raised against BP and was characterized initially as recognizing BP strongly but AMX or AMP relatively weakly when these drugs were conjugated to purified HSA at comparable hapten densities. Thus, the relatively weak recognition of AMP- and AMX-conjugated serum and cellular antigens probably reflects at least in part the selectivity of the antiserum. Nevertheless, the antiserum showed broad specificity in that it could detect to varying degrees binding of AMP, AMX, BP, CEP, CLX, FLX, and PMP to cells and serum by ELISA. The antiserum recognized AMX and AMP in serum-conjugated form more strongly than in albumin-conjugated form: this finding is consistent with the recent report that low molecular mass components of serum, which are not present in purified or dialysed serum protein preparations, facilitate protein binding of penicillin (7).

β -lactam antibiotics comprise a two-ring nucleus incorporating a β -lactam ring conjugated to either a five-membered (penicillins) or a six-membered (cephalosporins) second ring. The side chain that attaches

through the secondary amino group on the β -lactam ring defines the type of β -lactam, and determines its antibiotic profile and pharmacodynamic properties. Antibody responses to penicillins can be quite side-chain specific; for example, some allergic patients produce IgE that recognizes only certain penicillins (2, 20). The rabbit polyclonal antibody used in this study clearly has a reasonably broad antibiotic specificity and is able to recognize β -lactams differing in both side-chain and nuclear structure, but nevertheless is able to distinguish between different antibiotics in terms of their capacity to generate antigen. For example, antigens generated by BP, FLX and PMP, and the cephalosporin CEP were detected relatively strongly compared to AMX, AMP, and CLX. The differential antigen generation by FLX and CLX is of interest: both possess a relatively bulky five-membered ring structure between the β -lactam linkage and the side-chain benzene ring, but differ only in that FLX possesses a single fluorine substitution on the benzene ring of the side chain. Again, we cannot say for sure whether this minor structural difference influences the degree of conjugation or reflects the selectivity of the antiserum. The antiserum recognized CEP-derived antigen strongly: this is a cephalosporin which differs from the penicillins in the structure of both its side chain and nucleus. Quite clearly then, the polyclonal rabbit antiserum can recognize β -lactam-derived antigens that differ quite markedly in chemical structure, and on this basis we might speculate that there is a genuine difference in the capacity of FLX and CLX to generate antigen.

When the spleen cell and serum proteins were separated by SDS-PAGE and analysed by Western blotting, the relative capacity of the various antibiotics to generate positive immunostained protein bands correlated quite well with their capacity to generate detectable cell and serum antigens as seen by ELISA: BP, CEP, and PMP generated intense bands while AMX, AMP, and CLX generated very weak signals. The exception to this rule was FLX, which generated cell and serum antigens that were readily detected by ELISA, but not by Western blotting. FLX therefore appears to be atypical in that it generates an antigen that is lost on SDS-PAGE; presumably, the conjugated drug dissociates upon sample preparation or electrophoresis that involves boiling, denaturation, and reduction.

The three drugs that stained strongly on Western blots of cell and serum proteins, namely, BP, CEP, and PMP, produced very similar patterns of positive bands, and we saw no evidence that these compounds differ with regard to their target of the various proteins in cells or serum. However, patterns of protein immunostaining for each of these compounds did not reflect exactly the relative abundance

of total proteins in the samples, indicating some degree of protein selectivity of binding. For example, in both cell and serum samples, several protein bands that stained strongly with Coomassie brilliant blue were antigen-negative, while other bands that were antigen-positive did not stain strongly for protein. We have yet to establish the identity of the major cellular and serum protein targets for β -lactam conjugation. In serum samples, however, albumin would appear to be the major protein to which BP and the other compounds tested became conjugated, since an intensely stained band is present in all the drug-treated serum samples at a position of approximately 66 kDa. We recently reported that the penicillin-derived antiarthritic drug D-penicillamine binds *in vivo* by disulphide linkage to a plasma protein that migrates at the trailing edge of serum albumin on SDS-PAGE (15), and therefore binds to quite different serum protein targets by comparison with the antibiotics reported here. In the present study, in addition to the immunostained albumin band, a number of less intensely stained bands were also seen in the serum protein samples, but the identity and significance of these protein conjugates are as yet unknown. As for serum, we are as yet unable to identify the major spleen-cell protein bands detected after treatment with BP, CEP, or PMP. Evidence from *in vitro* T-cell proliferation studies suggests that penicillins can be presented only by autologous cells carrying MHC class II (1, 9), and it is conceivable that the band we observed at approximately 34 kDa may be a reduced component or fragment of this surface antigen.

Acknowledgment

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References

1. BELL SJD, PICHLER WJ. Penicillin-allergic patients react to penicillin-modified "self". *Allergy* 1989; 44: 199-203.
2. BLANCA M, MAYORGA C, SANCHEZ F, et al. Differences in serum IgE antibody activity to benzylpenicillin and amoxicillin measured by RAST in a group of penicillin allergic patients. *Allergy* 1991; 46: 632-8.
3. CHRISTIE G, COLEMAN JW, NEWBY S, et al. A survey of the prevalence of penicillin-specific IgG, IgM and IgE antibodies detected by ELISA and defined by hapten inhibition in pa-

- tients with suspected penicillin allergy and healthy controls. *Br J Clin Pharmacol* 1988; 25: 381-6.
4. COLEMAN JW. Allergic reactions to drugs: current concepts and problems. *Clin Exp Allergy* 1990; 20: 79-85.
5. DE WECK AL. Penicillins and cephalosporins. In: DE WECK AL, BUNDGAARD H, eds. *Allergic reactions to drugs. Handbook of experimental pharmacology*. Vol. 63. Berlin: Springer-Verlag, 1983: 423-82.
6. DE WECK AL, SPENGLER H, GECZY AF. Stimulation of penicillin-sensitive lymphocytes by antigenic determinants covalently bound to cell membranes. *Monogr Allergy* 1984; 8: 120-4.
7. DI PIRO JT, ADKINSON NF, HAMILTON RG. Facilitation of penicillin haptenation to serum proteins. *Antimicrob Agents Chemother* 1993; 37: 1463-7.
8. HACKENBECK R, BRIESE T, ELLERBROK H. Antibodies against the benzylpenicilloyl moiety as a probe for penicillin-binding proteins. *Eur J Biochem* 1986; 157: 101-6.
9. HERTL M, BOHLEN H, JUGERT F, BOECKER C, KNAUP R, MARK HF. Predominance of epidermal CD8⁺ lymphocytes in bulbous cutaneous reactions caused by beta-lactam antibiotics. *J Invest Dermatol* 1993; 101: 794-9.
10. IDSOE O, GUTHE T, WILLCOX RR, DE WECK AL. Nature and extent of penicillin side reactions, with particular reference to fatalities from anaphylactic shock. *Bull World Health Organ* 1968; 38: 159-88.
11. JUHLIN L, WIDE L. Detection of penicillin allergy by radioallergosorbent test. *Lancet* 1979; ii: 261.
12. KITTINGHAM NR, CHRISTIE G, COLEMAN JW, YEUNG JHK, PARK BK. Drug protein conjugates. XII. A study of the disposition, irreversible binding and immunogenicity of penicillin in the rat. *Biochem Pharmacol* 1987; 36: 601-8.
13. KOPONEN M, PICHLER WJ, DE WECK AL. T cell reactivity to penicillin: phenotypic analysis of *in vitro* activated cell subsets. *J Allergy Clin Immunol* 1986; 78: 645-6.
14. LAEMMLI UK. Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1987; 227: 680-5.
15. LAYCOCK CA, PHELAN MJI, BUCKNALL RC, COLEMAN JW. A Western blot approach to detection of human plasma protein conjugates derived from D-penicillamine. *Ann Rheum Dis* 1994; 53: 256-60.
16. O'DONNELL CA, FOSTER AL, COLEMAN JW. Penicillamine and penicillin can generate antigenic determinants on rat peritoneal cells *in vitro*. *Immunology* 1991; 72: 571-6.
17. PARK BK, COLEMAN JW, KITTINGHAM NR. Drug disposition and hypersensitivity. *Biochem Pharmacol* 1987; 36: 581-90.
18. SAXON A, BEALL GN, ROHR AS, ADELMAN DC. Immediate hypersensitivity reactions to beta-lactam antibiotics. *Ann Intern Med* 1987; 107: 205-15.
19. TOWBIN H, STAHLIN T, GORDON J. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 1980; 76: 4350-4.
20. WALLEY T, LEE S, COLEMAN J. A study of the frequency and crossreactivity of antibodies to benzylpenicillin and other beta-lactam antibiotics. *Br J Clin Pharmacol* 1991; 32: 663P-4.

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