

Basic aspects related to penicillin-allergy skin testing: on the variability of the hapten-paratope interaction

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Ampicillin and benzylpenicillin conjugated to human serum albumin were used as immunogens in order to obtain antihaptenic IgG responses in outbred guinea pigs according to different schedules, all involving complete Freund's adjuvant. The individual responses were characterized by ELISA and by ELISA inhibition using ampicillin, benzylpenicillin, and carbenicillin peptidic conjugates for coating and for inhibition. In several instances, drastically reduced cross-reactivity and even its absence were observed, although the penicillin antigens differ only in the side-chain. The notion that the invariantly present thiazolidine ring will always provide significant binding to antibodies against all penicillins differing only in the side-chain has to be dropped. The experiments were performed in relation to newer findings of clinical penicillin-allergy skin testing which suggest that benzylpenicillin-based reagents alone are not able to detect or predict all reactions against semisynthetic penicillins. The experimental evidence here obtained corroborates this conclusion.

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It is widely accepted that skin testing with appropriate reagents is a simple and reliable method for detecting individuals potentially exhibiting immediate-type reactions after penicillin administration. This view is based on a number of large-scale studies which used polymeric benzylpenicilloyl conjugates as well as MDM, a so-called minor determinant mixture. MDM, which detects about 10% of reactors negative with the multivalent reagents, includes benzylpenicillin and its hydrolysis product benzylpenicilloic acid (23, 25) and in some instances also penilloic acid (1, 7, 9, 13, 14) or a monovalent penicilloylamide (13, 23). All reagents generally include the benzyl side-chain, and this applies also to the commercially available test kit (Allergopharma, Reinbek b. Hamburg), which involves an optimized oligovalent penicilloyl conjugate.

The justification for this approach is based on the notion that all penicillins differ only in the side-chain and have a prominent structural moiety, the thiazolidine ring, in common. Immunochemical studies

have demonstrated the importance of the thiazolidine moiety in a rabbit polyclonal response against benzylpenicilloyl (3). In a group of rabbits, some individual variation in the proportion of antibodies reacting with the thiazolidine moiety could be seen, but this variation was not large and no discussion pertinent to this aspect was offered (2). Furthermore, some early patient studies specifically concluded that benzylpenicillin-based reagents detect most or virtually all clinically significant allergic reactivity to all penicillins (8, 13). This is also reflected by the statement that for cutaneous reactivity no compelling need can be seen for the use of antigenic determinants other than those derived from benzylpenicillin (7). All this evidence seemed to justify the view that the invariant thiazolidine would provide sufficient interactions with all antibody populations raised against penicillin antigens, and that a lack of cross-reactivity between different side-chains would therefore not be diagnostically important.

While the multivalent reagents initiate immediate-type reactions by cross-linking membrane-bound specific IgE on vasoactive cells, it should be kept in mind that the mechanistic as well as the specificity aspects of the reactions seen with penicilloic acid

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and other MDM reagents are not so clearly defined (11, 18).

Some years ago, clinical reports appeared to imply that immediate-type penicillin reactions may be predominantly or even exclusively side-chain specific in a proportion of patients who tolerated benzylpenicillin but reacted to amoxicillin (5, 6). In another study, among a large group of penicillin-allergic patients, the inclusion of ampicillin- and methicillin-based reagents resulted in a larger number of reactors than when benzylpenicillin-based reagents alone were used (24). Moreover, in very recent contributions, ampicillin, amoxicillin, or minor determinant mixtures from them detected significant proportions of reactions which were not found with benzylpenicillin reagents, again suggesting the involvement of side-chain specific interactions (4, 22).

Taking into account this clinical background, we set out to find variations of haptenic cross-reactivity by studying antisera from individual outbred guinea pigs immunized with ampicilloyl- or benzylpenicilloyl-protein, using ELISA and ELISA inhibition for assessment.

Material and methods

Immunogens (cf. Fig. 1)

Benzylpenicilloyl-HSA (Bpo-HSA). Penicilloylation of 400 mg HSA (Fluka, Buchs) was carried out with 400 mg benzylpenicillin sodium salt in K_2CO_3 solution at pH 10 overnight at ambient temperature (cf. Reference 17). The solution was neutralized (HCl) and lyophilized, and the lyophilizate chromatographed with 0.05 M PBS, pH 7.4, on a Sephadex G-25 (3×48 cm) column. The protein peak emerging first was rerun on the column with H_2O in order to desalt the preparation, which was then lyophilized. The penamaldate assay (15) indicated a substitution of 26 Bpo-groups per conjugate molecule.

Ampicilloyl-HSA (Amp-HSA). This was prepared by similar penicilloylation using ampicillin with its side-chain amino group protected by a tert. butoxycarbonyl residue (Boc-Amp). The lyophilized, desalted Boc-Amp-HSA (300 mg) was stirred for 40 min with 4 ml 20% trifluoroacetic acid at 0° , mixed with 5 ml H_2O , neutralized with 1 M NaOH, and lyophilized. The lyophilizate was taken up in 20 ml 0.002 M phosphate buffer, pH 7.4, and subjected to membrane filtration in an Amicon cell S-43-50 (Amicon, Danvers, MD, USA) with a YM 3 membrane, of 43-mm diameter, and molecular weight cutoff of 3000, using N_2 at a pressure of 2 atm and a flow rate of 20 ml/h. The volume was kept constant with buffer for 18 h and with H_2O for 2 h. Lyophilization gave 120 mg of product containing 22 ampicilloyl groups per protein molecule (penamaldate assay). Complete removal of the Boc-protection under the above conditions was ascertained in a control experiment with Boc-Amp first reacted with 6-aminohexanoic acid. The removal of Boc could be followed by thin-layer chromatography.

Boc-ampicillin. Ampicillin trihydrate (5.0 g; 12.4 mmol) suspended in 50 ml dioxan was mixed with 1.36 ml (12.4 mmol) *N*-methylmorpholine and then with 2.97 g (13.6 mmol) di-tert.-butyldicarbonate (Fluka, Buchs) within 1 h. The suspension was stirred at ambient temperature until a clear solution was obtained after 5 h. Lyophilization gave 6.70 g of a solvent-containing product. Thin-layer chromatography (ethylacetate/acetic acid/ H_2O , 3:1:1): R_F 0.98, homogeneous; (dioxan/ H_2O , 95:5): R_F 0.65, homogeneous (UV +, ammoniacal $AgNO_3$ +; silica gel 60, F 254 plates, Merck, Darmstadt, Germany). Elementary analysis after drying at the oil pump over P_4O_{10} at $37^\circ C$ was as follows:

Calcd. C 56.12 H 6.01 N 9.35 S 7.12%; found C 55.81 H 6.25 N 9.54 S 6.86%.

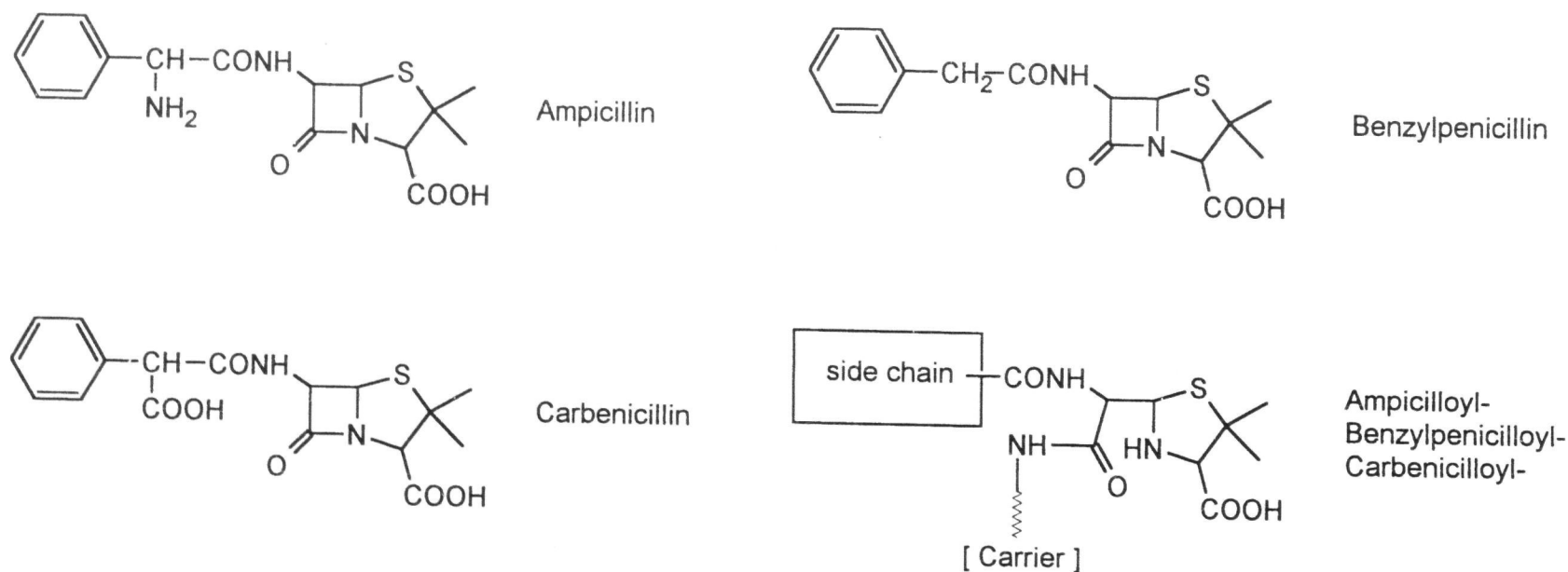


Fig. 1. Chemical structures of ampicillin, benzylpenicillin, and carbenicillin with their penicilloyl haptenic moieties.

Penicillin antigens for ELISA. Poly-L-lysine (PLL), chain length $n = 20$, was penicilloylated with benzylpenicillin and in another run with carbenicillin. Conditions for penicilloylation, chromatography, and desalting were similar to the procedure for Bpo-HSA. The molar ratio of penicillin/lysine free amino groups was set at 0.9:1 in order to give conjugates Bpo-PLL and Cab-PLL a degree of substitution of 60–70%. The remaining unsubstituted amino groups were required for anchoring the antigens on the ELISA plates during coating.

This precaution was unnecessary for ampicilloyl-PLL. The conjugate Amp-PLL was obtained by penicilloylation of all 10 amino groups of a decalysine peptide (16) with Boc-ampicillin, followed by Boc removal from the conjugate. This reaction took place in 80% trifluoroacetic acid at 0° for 1 h. After removal of the bulk of the reagent *in vacuo*, the residue was taken up in H₂O, neutralized with 1 M NaOH, and lyophilized. Chromatography and desalting were carried out on a Biogel P-6 DG column. Degrees of substitution were controlled in all cases by the penamaldate assay (15).

We have previously used in several instances, as in other studies (12, 19), direct attachment of haptens to ELISA plates not involving a protein carrier in order to benefit from well-defined specificities of interaction. We feel that the use of multiple-haptenated polylysines is even better because, due to a cluster effect, enhanced affinities come into play (20).

ELISA. The method involving coating of amino compounds and peptides to microtiter plates was described previously (12, 26). Alkaline phosphatase-conjugated goat anti-guinea pig IgG (H + L) (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was used. For inhibition experiments, antiserum plus inhibitor were preincubated in glass tubes at ambient temperature for 4 h. The titer is defined as the dilution factor which results in an absorbance of 1.0.

Both measurements of titers and inhibition assessments were performed with the same reagents within a few weeks. In a number of instances, the same antiserum was reassessed at a different date. The duplicated data were within reasonable agreement.

Immunizations

GOHI-guinea pigs (outbred, female, 250 g) were obtained from BRL, Füllinsdorf, Switzerland. Three schedules for immunization were used, differing in the amounts of immunogen and the number of boosts. Primary injections were made subcutaneously, boosts intradermally.

Schedule I. An amount of 1 mg HSA conjugate in 0.5 ml PBS (0.01 M, pH 7.4) plus 0.5 ml complete Freund's adjuvant (CFA) was emulsified for injection. Boosts of 0.1 mg conjugate in 0.1 ml PBS were done after 2, 4, 6 and 7 weeks. Bleedings for antisera were done after 8 weeks.

Schedule II. An amount of 0.05 mg conjugate in 0.1 ml PBS plus 0.1 ml CFA was prepared. Boosts of 0.005 mg conjugate in 0.1 ml PBS were done after 2 and 4 weeks. Bleedings for antisera were done after 5 weeks.

Schedule III. An amount of 10 mg conjugate in 1 ml PBS plus 1 ml CFA was prepared. Boosts of 1 mg conjugate in 0.1 ml PBS were done after 2 and 4 weeks. Bleedings for antisera were done after 5 weeks.

Results

Synthetic aspects

Ampicillin (Fig. 1) with its free amino group in the side-chain undergoes self-condensation to give ampicilloyl-ampicillin and higher oligomers under the conditions of penicilloylation in alkali. To avoid the complication of oligomeric ampicilloyl antigenic determinants on the immunogens as well as on the peptide conjugates, we chose to protect the amino function during the ampicillin reaction with the Boc-protective group, which can later be removed under mild conditions with trifluoroacetic acid. This is a standard procedure in peptide chemistry, and it may be of interest to note that it has worked quite well also during the preparation of the protein conjugate Amp-HSA.

ELISA titers

The titers measured with the homologous haptenic coat were similar in six out of eight Amp-HSA immunized animals, and were about two orders of magnitude higher, but again with little variation, in the Bpo-HSA-immunized animals. The schedule of immunization seems to have no marked effect on the titers (Table I). The Amp-HSA-immunized animals 38A and 39A had titers which are about one order of magnitude higher than the rest of Amp-HSA animals. On the other hand, these animals had particularly low titers when measured with the Bpo-coat; i.e., the Amp-coat gave a 54.44 and a 43.46 times higher value than the Bpo-coat. All other titer ratios were less remarkable although some variation was seen.

Table 1. ELISA titers of Amp- and BPO-immunized guinea pigs with Amp-, Bpo-, and Car-coated plates

Immunization	Animal no.	Titers	Titer ratios	
		<i>Amp-coat</i>	<i>Amp-coat</i> Bpo-coat	<i>Amp-coat</i> Car-coat
<i>Amp-HSA</i>				
Schedule I	38A	3.49×10 ³	54.44	3.32
	39A	3.65×10 ³	42.46	2.38
	40A	4.73×10 ²	1.69	1.47
	41A	3.27×10 ²	4.43	2.18
Schedule II	50D	4.61×10 ²	1.62	1.05
	51D	4.57×10 ²	1.29	1.02
	52D	2.90×10 ²	1.71	1.15
Schedule III	55E	3.10×10 ²	2.44	0.88
	56E	3.73×10 ²	1.64	1.02
Normal serum	37N	< 10		
<i>Bpo-coat</i>				
<i>Bpo/Amp</i>				
<i>Bpo/Car</i>				
<i>Bpo-HSA</i>				
Schedule I	41B	4.01×10 ⁴	4.89	8.90
	42B	4.84×10 ⁴	5.72	4.65
	43B	4.67×10 ⁴	4.18	7.13
	44B	5.20×10 ⁴	2.31	8.71

Inhibition of ELISA

It is to be noted that in instances with marked inhibition ($\log I/IC_{50}$ around 6), full inhibition at higher inhibitor concentration was generally found, as shown with two examples in Fig. 2. Homologous

Table 2. Inhibition of ELISA by Amp-, Bpo-, and Car-antigens^a

Animal no. ^b	Coat specificity	Antiserum specificity	Inhibition ($\log 1/IC_{50}$) with:		
			Amp-	Bpo-	Car-antigen
38A	Amp	anti-Amp	Not done	4.2	4.5
39A			6.8	3.5	3.0
40A			6.6	5.8	< 1
41A			6.0	5.3	< 1
50D	Amp	anti-Amp	7.2	4.6	6.0
51D			5.8	5.4	4.3
52D			5.8	3.6	< 1
53D			4.7	3.2	< 1
54D	Amp	anti-Amp	6.3	4.8	3
55E			5.4	4.1	< 1
56E			5.4	4.0	3.2
42B	Bpo	anti-Bpo	4.4	5.2	5.5
43B			1.5	6.2	6.8
44B			< 1	6.5	3.8
45B			1.0	7.0	5.4

^a The same penicillin antigens used for coating the ELISA plates were used for haptenic inhibition. Triplicate measurements were used to draw the inhibition curve from which the molar inhibition concentration giving 50% inhibition was read. Molar concentrations are based on the conjugate molecular masses and do not therefore take into account the number of haptenic groups per conjugate molecule. The "haptenic concentration" would be around 10 times higher depending on the degree of substitution and the chain lengths of the polylysines.

^b From the animal number, the schedule of immunization can be found in Table 1. Animals 53D and 54D, not included in Table 1, were immunized according to schedule II.

inhibition, i.e., Amp-inhibited interaction of Amp/anti-Amp and Bpo-inhibited interaction of Bpo/anti-Bpo, was usually around 6 ($\log I/IC_{50}$) with values going up to 7.2 and down to 4.7 (Table 2).

The Bpo-inhibition of Amp/anti-Amp was consistently lower than the homologous inhibition, but not uniformly so. In 39A of Table 2, the difference is more than three orders of magnitude (6.8 versus 3.5), whereas 51D, for example, showed little difference (5.8 versus 5.4). It is clear that considerable variation of cross-reactivities was involved. This is most striking with Cab-inhibitors where, in a number of instances, no significant inhibition was found (values <1), whereas in some other cases strong inhibition was also present. Among the interesting examples are 40A and 41A where the Amp/anti-Amp interaction, strongly inhibited by the homologous inhibitor, was still strongly inhibited by the Bpo-inhibitor, but insignificantly so by Cab. The Bpo/anti-Bpo interaction was well inhibited by Cab in the four instances studied, whereas Amp was a weak or insignificant inhibitor in three out of the four cases of Table 2.

Discussion

Progress in peptide methodology and in particular the development of microscale epitope-mapping

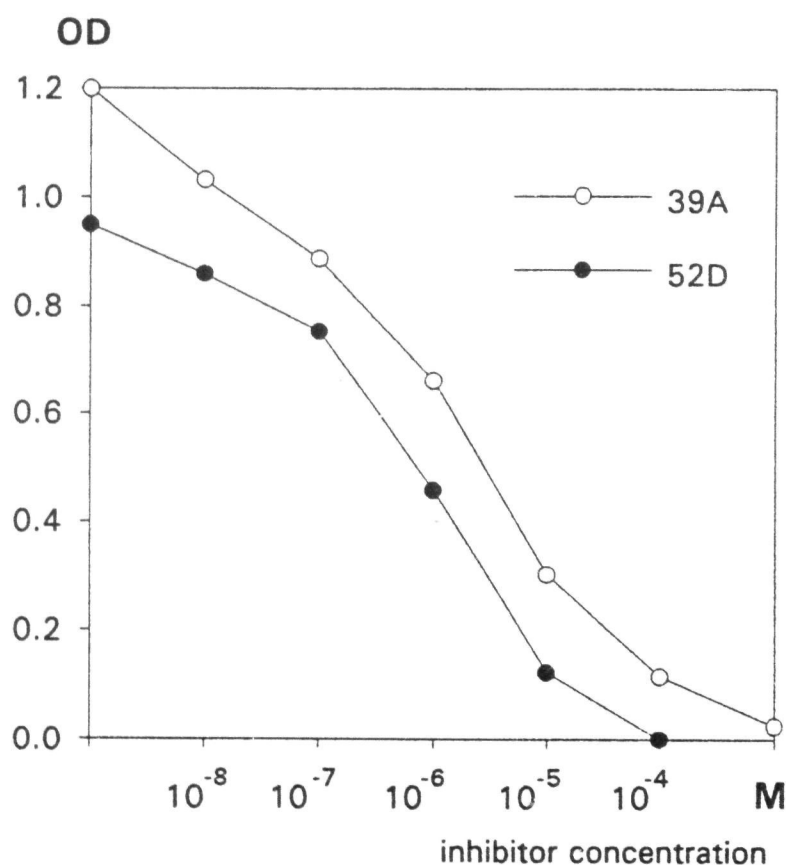


Fig. 2. ELISA inhibition curves. Anti-Amp antisera 39A and 52D were inhibited with Amp-peptide in Amp-coated plates. Coating concentration of peptides was in all cases 10^{-6} M.

techniques have allowed new insights into the parameters of protein antigenic determinants, or epitopes, as they are now more frequently called. These epitopes comprise not only five or six amino acids in a chain but up to 20 or 25 units which may come from different but spatially vicinal domains. Furthermore, there may be drastic individual variations; i.e., the main epitopes recognized by one animal may be quite different from those recognized by another (10).

Whether drastic individual variation may also occur in the antibody response against haptens, i.e., small drug- or chemical-derived residues covalently bound to protein, has apparently not been discussed so far. We dismiss the notion of special paratopes comprising only a small contact area comparable in size to the hapten, and assume that the same type of paratopes are selected at the B-cell level that are involved in antiprotein responses. The common denominator of hapten-specific paratopes would be a sublocus within the contact area with the capacity to interact strongly with the hapten or parts of it. From this, it follows that many quite different paratopes could become involved, their different overall specificity not becoming apparent in haptenic bindings.

Indeed, when a bovine gamma globulin conjugate with three different haptenic groups was studied in outbred guinea pigs, the response profile against the three haptens remained the same in a large number of animals. Furthermore, cross-reactivity patterns were essentially regular with only slight variations. Thus far, the data have been published only in part (21).

Therefore, it seems fortunate that within a small number of animals quite drastic examples of variation and lack of cross-reactivity could be found, the reason perhaps being related to the fact that penicilloyl haptens are not small. The drastic variations are mostly found in the inhibitor series with carbenicillin antigen in Table 2, and thus are not directly related to the clinical findings described at the beginning of this paper where a lack of cross-reactivity between ampicillin and benzylpenicillin reactions is involved. The anti-Amp/Amp interaction is not well inhibited in a number of cases, e.g., 39A, 52D, and 53D of Table 2, but the data are not as impressive as the clinical results may suggest. On the other hand, the anti-Bpo/Bpo interaction is definitely not well inhibited in three out of four cases. However, the anti-Amp antisera 38A and 39A of Table 1, which, respectively, show a 54 and 43 times lower binding with Bpo compared to the homologous binding, point in the direction of the clinical results.

In any case, what is demonstrated by the present data is that chemical modification of the penicillin side-chain may drastically reduce, if not abolish, the capacity of specific interaction in individual anti-

body responses. It follows that the notion related to the thiazolidine moiety, which as an invariant structural feature would regularly provide a sufficient basis for specific penicillin/antipenicillin interaction irrespective of the side-chain, has to be dropped. To make this issue very clear, we may add that the thiazolidine moiety as such remains as important as ever, but does not regularly apply in every individual case as was previously thought by many. We deal here with what amounts to a change in paradigm.

The practical implications are not to be discussed here at length. Suffice it to say that, if one wants to maintain virtually error-free skin testing, it is necessary to test with the semisynthetic penicillin actually to be administered. This will minimally involve the penicillin itself, probably its penicilloic acid, and certainly its multivalent penicilloyl conjugate.

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References

- ADKINSON NF, THOMPSON WL, MADDREY WC, LICHTENSTEIN LM. Routine use of penicillin skin testing on an inpatient service. *N Engl J Med* 1971; 285: 22-4.
- ATSUMI T, ADACHI M, KINOSHITA Y, KAWASAKI M, HORIUCHI Y. The heterogeneity of combining sites of anti-benzylpenicilloyl antibodies obtained from individual rabbits: changes in combining sites of gammaG and gammaM antibodies during the immune response. *J Immunol* 1968; 101: 1016-22.
- ATSUMI T, NISHIDA K, KINOSHITA Y, SHIBATA K, HORIUCHI Y. The heterogeneity of combining sites of anti-benzylpenicilloyl antibodies obtained from individual rabbits. Fractionation of antibodies with specific immunosorbent. *J Immunol* 1967; 99: 1286-93.
- AUDICANA M, BERNAOLA G, URRUTIA I, et al. Allergic reactions to betalactams: studies in a group of patients allergic to penicillin and evaluation of cross-reactivity with cephalosporin. *Allergy* 1994; 49: 108-13.
- BLANCA M, PEREZ E, GARCIA J, et al. Anaphylaxis to amoxicillin but good tolerance for benzylpenicillin. *Allergy* 1988; 43: 508-10.
- BLANCA M, VEGA JM, GARCIA J, et al. Allergy to penicillin with good tolerance to other penicillins; study of the incidence in subjects allergic to betalactams. *Clin Exp Allergy* 1990; 20: 475-81.
- CHANDRA RK, JOGLEKAR SA, TOMAS E. Penicillin allergy: anti-penicillin IgE antibodies and immediate hypersensitivity skin reactions employing major and minor determinants of penicillin. *Arch Dis Child* 1980; 55: 857-60.
- DESWARTE RD. Drug allergy problems and strategies. *J Allergy Clin Immunol* 1984; 74: 209-21.
- GADDE J, SPENCE M, WHEELER B, ADKINSON F. Clinical experience with penicillin skin testing in a large inner-city STD clinic. *JAMA* 1993; 270: 2456-63.
- GEYSEN HM, RODDA SJ, MASON TJ. The delineation of peptides able to mimic assembled epitopes. In: Ciba Foundation Symposium 119. Synthetic peptides as antigens. Chichester: Wiley, 1986: 130-49.

11. GUENIN R, SCHNEIDER CH. Penicillin derivatives are true monovalent elicitors of anaphylactic reactions. *Immunology* 1984; 52: 189-96.
12. KASPER MF, SCHNEIDER CH, ROLLI H, ANGST BD, DE WECK AL. Diagnostic reagents in drug allergy: immunochemical specificity in the 1,2-diphenyl-pyrazolidinedione series. *Immunobiology* 1986; 173: 98-109.
13. LEVINE BB, ZOLOV DM. Prediction of penicillin allergy by immunological tests. *J Allergy* 1969; 43: 231-44.
14. MENDELSON LM, RESSLER C, ROSEN JP, SELCOW JE. Routine elective penicillin allergy skin testing in children and adolescents: study of sensitization. *J Allergy Clin Immunol* 1984; 73: 76-81.
15. ROLLI H, SCHNEIDER CH. Penamaldate assay: improved methodology for the quantitation of penicilloic acids, penicilloyl amides and penicilloyl esters. *Arzneimittelforschung/Drug Res* 1984; 34: 1247-9.
16. ROLLI H, SCHNEIDER CH. Peptide conjugates as diagnostic reagents in clinical immunology. In: THEODOROPoulos D, ed. *Peptides* 1986. Berlin: de Gruyter, 1987: 543-7.
17. SCHNEIDER CH, DE WECK AL. Chemische Aspekte der Penicillin-Allergie: Die direkte Penicilloylierung von epsilon-Aminogruppen durch Penicilline bei pH 7.4. *Helv Chim Acta* 1966; 49: 1695-1707.
18. SCHNEIDER CH, GUENIN R, TOFFLER O. Immediate hypersensitivity to drugs and simple chemicals: the efficacy of monovalent elicitors. *Eur J Immunol* 1985; 15: 155-62.
19. SCHNEIDER CH, KASPER MF, DE WECK AL, ROLLI H, ANGST BD. Diagnosis of antibody-mediated drug allergy. *Allergy* 1987; 42: 597-603.
20. SCHNEIDER CH, ROLLI H. Potency of hexavalent and decavalent anaphylactogens: affinity enhancement by epitope-clusters. *Immunobiology* 1988; 178: 250-60.
21. SCHNEIDER T, ZHAO Z, SCHNEIDER CH. Haptenic conjugates of peptides and proteins: a comparative study of immunogenicity. In: SMITH JA, RIVIER JE, eds. *Proceedings of the Twelfth American Peptide Symposium*. Leiden: Escom, 1992: 863-4.
22. SILVIU-DAN F, MCPHILLIPS S, WARRINGTON RJ. Clinical aspects of allergic disease. The frequency of skin test reactions to side-chain penicillin determinants. *J Allergy Clin Immunol* 1993; 91: 694-701.
23. SOGN DD, EVANS R, SHEPHERD GM, et al. Results of the National Institute of Allergy and Infectious Diseases collaborative clinical trial to test predictive value of skin testing with major and minor penicillin derivatives in hospitalized adults. *Arch Intern Med* 1992; 152: 1025-32.
24. SOLLEY GO, GLEICH GJ, VAN DELLEN RG. Penicillin allergy: clinical experience with a battery of skin-test reagents. *J Allergy Clin Immunol* 1982; 69: 238-44.
25. SULLIVAN TJ, WEDNER HJ, SHATZ GS, YECIES LD, PARKER CW. Skin testing to detect penicillin allergy. *J Allergy Clin Immunol* 1981; 68: 171-80.
26. VON GRÜNIGEN R, SCHNEIDER CH. Antigenic structure of the hexacosapeptide melittin: evidence for three determinants, one with a helical conformation. *Immunology* 1989; 66: 339-42.

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