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Penicillin Allergy and the Heterogeneous Immune Responses of Man to Benzylpenicillin *

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The administration of benzylpenicillin (penicillin) to man has been shown to induce the synthesis of antibodies of several distinct haptenic specificities and of several molecular classes (1–13). Specifically, skin-sensitizing antibodies of benzylpenicilloyl (BPO) haptenic specificity (1–7, 11) and of the minor determinant specificities (6, 7, 11) have been demonstrated. Also, BPO-specific antibodies of IgM and IgG classes have been identified in serum by passive hemagglutination (3–5, 8–10, 12, 13). We now report the results of assays for BPO-specific IgM, IgG, and skin-sensitizing antibodies, and minor determinant specific skin-sensitizing antibodies in various groups of patients with and without recent allergic reactions to penicillin. The data obtained describe the heterogeneous immune responses to penicillin made by human beings and permit initial inferences as to the nature of the immunological mechanisms that mediate allergic reactions to penicillin.[1]

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[1] These results are limited to benzylpenicillin, and do not necessarily apply to the semisynthetic penicillins. Immune responses to semisynthetic penicillins might differ depending on differences among the various penicillins in chemical reactivity and in their "intrinsic antigenicities" relating to the chemical structures of their side chains. Penicillins cross react allergically to large but varying extents.

Methods

Patients. The 302 patients in this study ranged from 22 to 80 years of age and were of both sexes. The 60 patients in group I were from a private medical clinic in New York City. These patients were generally in good health. They were professional people and gave reliable medical histories. The other 242 patients were from the wards of Bellevue Hospital. All the ward patients had acute infectious diseases requiring penicillin therapy; many were also chronically ill. None had lymphoproliferative disease. Most gave reasonably reliable medical histories. Almost all patients treated with penicillin were treated simultaneously with several other drugs. Adverse drug reactions were diagnosed as immediate, accelerated, or late allergic reactions on the basis of the clinical criteria described in Results. Patients were generally clinically evaluated by two of the authors. The clinical diagnosis was made before the immunological studies were done.

Immunological studies. Skin-sensitizing antibodies [2] were assayed by direct skin tests for wheal and flare reactions and in some cases also by passive transfer (6). Three skin tests were done: *a*) 1×10^{6} M BPO_{13}-$PLL_{20}S$ (BPO-polylysine preparation of 20 lysine residues, 13 coupled with BPO and the others succinylated; see reference 15); *b*) 1×10^{-2} M potassium benzylpenicillin (penicillin)[3]; and *c*) 1×10^{-2} M crystalline sodium benzylpenicilloate.[4] These reagents were prepared, diluted, and stored as described previously (15, 17). Volumes of about 0.005 ml were injected intradermally into the deltoid area. Positive reactions were typical wheal and flares read at 15 minutes; negative reactions were 1- to 2-mm blebs. The materials were nonirritating as used. Previous studies showed these reagents and the

[2] IgG antibodies are 7 S (molecular weight about 160,000), and IgM antibodies are 19 S (molecular weight about 1,000,000). Skin-sensitizing antibodies appear to be neither IgM nor IgG antibodies. The antibody class (or subclass) to which they belong has not yet been clearly determined. Recently, Ishizaka, Ishizaka, and Hornbrook have suggested that they belong to a new major class, termed IgE (14).

[3] Generously provided by Bristol Laboratories, Syracuse, N. Y.

[4] Prepared by alkaline hydrolysis of sodium benzylpenicillin (16).

concentrations used to be presently the optimal elicitors of wheal and flare reactions (15, 18) in the penicillin system. BPO-polylysine detects BPO-specific skin-sensitizing antibodies, and penicillin and sodium benzylpenicilloate detect minor determinant-specific skin-sensitizing antibodies [5] (see reference 6 for hapten inhibition data). Serum assays for skin-sensitizing antibodies were done by passive transfer to the skin of nonsensitive human recipients with local challenge with the three reagents after a 48-hour latent period (6). In virtually all cases where skin-sensitizing antibodies could be detected in the serum, they could also be detected by direct skin test. The few exceptions occurred in situations where penicillin therapy had been discontinued 1 to 2 days previously. In these exceptional cases, skin tests also became positive within 24 to 48 hours. Antihistamines were discontinued for at least 24 hours before recordable skin tests were done.

BPO-specific IgG and IgM antibodies.[2] Sera taken within 48 hours after penicillin administration were dialyzed at 4° C against three changes of buffered saline to remove penicillin. This treatment did not cause inactivation of antibodies. Sera were neither heat-inactivated, decomplemented, nor absorbed. They were stored nonsterile and without preservative at −20° C for up to 6 months before assay. At most, titers of IgM antibodies might have decreased by one tube during storage, but extensive degradation of antibodies was not found. Antibody assays were done by passive hemagglutination as described previously (12, 13). Briefly, O^+ human red blood cells (RBC) are passively sensitized by reaction with penicillin under controlled conditions. This procedure appears to covalently couple BPO groups directly to the RBC surface membrane (12). Serum dilutions (in diluent containing dextran and fetal calf serum) are incubated with sensitized RBC. The hemagglutination titer is the highest serum dilution giving agglutination. BPO specificity of hemagglutination is routinely confirmed by specific inhibition with a univalent BPO hapten, BPO-propylamine. Previous studies (12, 13) indicate that hemagglutination titers are a measure of antibody concentrations. IgG and IgM antibodies are distinguished by noting the effect of 0.1 M mercaptoethanol treatment on antibody titers. Prior studies have shown that sera whose titers are reduced to zero by treatment with mercaptoethanol contain virtually only IgM antibodies (termed "IgM sera"). Sera whose titers are not reduced by mercaptoethanol treatment contain mainly IgG antibodies (termed "IgG sera"). Sera whose titers are partially reduced by mercaptoethanol contain IgM and comparatively lower titers of IgG antibodies (13). IgG sera have been shown to also contain comparatively low concentrations of BPO-specific antibodies of IgM and other classes (probably including IgA) (13). These antibodies will not be considered in the present report. Low titers of mercaptoethanol sensitive antibodies cannot be detected in high-titered IgG sera by noting differences in titer before and after mercaptoethanol treatment. Serum antibody concentrations are expressed as the reciprocal hemagglutination titers taken, and after mercaptoethanol treatment, e.g., 4,096/0, 4,096/4,096, 4,096/256 indicate 1/4,096 IgM, 1/4,096 IgG (also containing low concentrations of IgM and other antibody classes, see above), and 1/4,096 IgM with 1/256 IgG. Sensitivity of the hemagglutination method is in the order of 0.0005 μg per ml of anti-BPO antibody protein (for IgG) (13).

[5] BPO-specific skin-sensitizing antibodies and the skin sensitizing-antibodies of the minor determinant specificities detected by sodium benzylpenicilloate appear to be distinct antibodies. Three sera containing both these skin-sensitizing antibodies were absorbed with a BPO-specific insoluble immunoabsorbant, which removed the BPO-specific skin-sensitizing antibodies but not the skin-sensitizing antibodies of the minor determinant specificities. Absorpton of the BPO-specific skin-sensitizing antibodies was specific, as these antibodies were not removed by a homologous dinitrophenyl-specific immunoabsorbant.

TABLE I

*Antibody assays in patients who did not have recent allergic reactions to PG**

Antibody pattern detected†	Percentage of patients having antibody pattern			
	Group 1, 60 clinic patients with no recent PG therapy	Group 2, 57 ward patients with no recent PG therapy	Group 3, 103 ward patients, recent PG therapy without allergic reactions‡	Group 4, 12 ward patients, recent high dosage PG therapy without allergic reactions‡
None	3	2	0	0
IgM alone (1:4 to 1:1,024)	81	73	61	92
IgM (1:128 to 1:1,024) plus lower titer of IgG (1:32 to 1:128)	5	5	11	0
IgG	8	15	19	8
SSA(BPO) plus IgG plus IgM	3	5	8	0
SSA(minor) plus SSA(BPO) plus IgG plus IgM	0	0	1	0

* Abbreviations: PG, benzylpenicillin; SSA(BPO), BPO-specific skin-sensitizing antibodies; SSA(minor), skin-sensitizing antibodies of minor determinant specificities.

† IgM and IgG antibodies are BPO specific.

‡ Assayed 7 to 14 days after penicillin therapy was discontinued.

Results

Antibody assays in patients who had not had recent penicillin therapy. Group 1 consisted of 60 consecutive patients from a private medical clinic in New York City. All denied having had penicillin therapy for at least 2 years, and 18 denied ever having penicillin therapy. Two of the 60 had past histories of penicillin allergy, 3 and 6 years previously. Table I shows that 97% of these patients had BPO-specific antibodies. Eighty-one per cent had only IgM antibodies; 13% had IgG, in addition to IgM, antibodies; and 3% had BPO-specific skin-sensitizing antibodies, in addition to IgM and IgG antibodies. Group 2 consisted of 57 consecutive patients selected at random from the wards of Bellevue Hospital. They denied having had penicillin therapy for at least 2 years. Five had past histories of penicillin allergy (four late reactions, one immediate reaction, 3 to 15 years previously). Table I shows that 98% of the patients in group 2 had antipenicillin antibodies. Seventy-three per cent had only IgM antibodies; 20% had IgG antibodies, in addition to IgM; and 5% had BPO-specific skin-sensitizing antibodies, in addition to IgG and IgM antibodies. None of the 117 patients in groups 1 and 2 had skin-sensitizing antibodies of minor determinant specificities. The distributions of hemagglutination titers of these patients are shown in Figure 1 A. The mean geometric titers were 1:32 for IgM and 1:256 for IgG antibodies.

Antibody assays in patients with recent penicillin therapy without allergic reaction. This group consisted of 103 consecutive ward patients who had recently completed courses of penicillin therapy without allergic reaction. Most had intramuscular procaine penicillin for 5 to 14 days in doses ranging from 600,000 to 2.4 million U per day. Antibody assays were done between the seventh and fourteenth days after penicillin therapy had been discontinued. Table I (group 3) shows that 100% of these patients had antipenicillin antibodies. Sixty-one per cent had only IgM antibodies; 30% had IgG antibodies, in addition to IgM; 8% had BPO-specific skin-sensitizing antibodies, in addition to IgG and IgM antibodies; and one patient had skin-sensitizing antibodies of minor determinant specificity, in addition to BPO-specific skin-sensitizing antibodies and IgM and IgG antibodies. The distribution of hemagglutination titers is shown in Figure 1, B. The mean geometric titers were 1:64 for IgM and 1:1,024 for IgG.

Immune response to penicillin in high dosage. This group consisted of 12 consecutive ward pa-

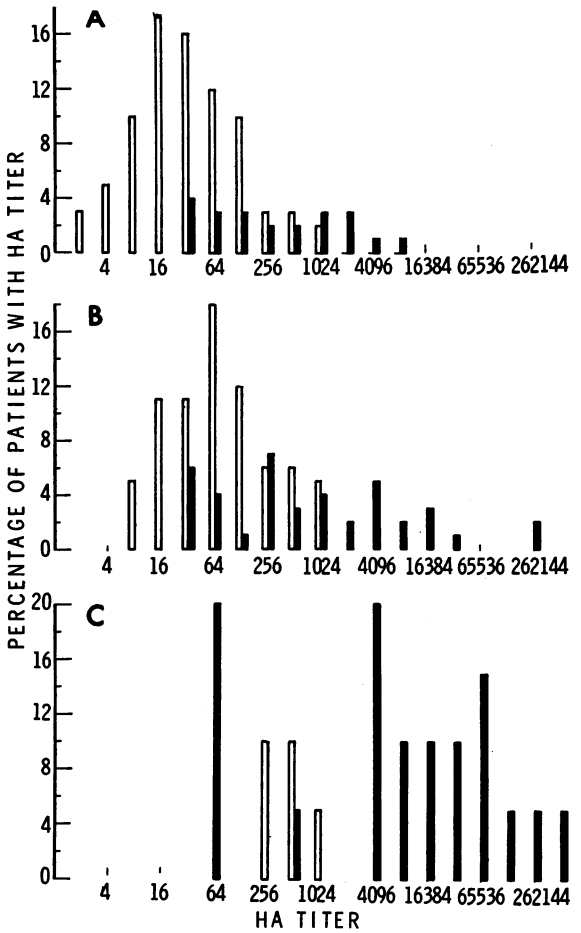

FIG. 1. HEMAGGLUTINATION (HA) TITERS OF VARIOUS GROUPS OF PATIENTS. Open bars represent titers of IgM antibodies, solid bars represent titers of IgG antibodies. In several patients, hemagglutination titers were partially reduced by mercaptoethanol treatment, i.e., they had comparatively lower titers of IgG antibodies (1:32 to 1:256) as well as higher titers of IgM antibodies (1:128 to 1:1,024). They were tabulated twice, as IgM and IgG. There were 6 of these patients in group A, 11 in group B, and 5 in group C. Group A consisted of 117 patients with no recent penicillin therapy. Group B consisted of 103 patients who had recently completed courses of penicillin therapy without allergic reactions. Assays were done 7 to 14 days after penicillin therapy had been discontinued. Group C included 21 patients that had accelerated (10) and late (11) urticarial reactions to penicillin therapy. Hemagglutination titers were of sera taken between 7 and 14 days after penicillin therapy had been discontinued. There was no significant difference in titers between late urticarial and accelerated reactors.

tients who were treated with 20 to 40 million U of penicillin per day intravenously for 3 to 6 weeks. None had allergic reactions to therapy. Antibody assays were done 7 to 14 days after benzylpenicillin (PG) was discontinued. Table I (group 4) shows that all 12 had IgM antibodies, but that only one patient had IgG antibodies in addition to IgM, and none had skin-sensitizing antibodies. Antibody titers were not significantly different from the preceding group (Figure 1, B). IgM titers ranged from 16 to 512 (geometric mean titer, 1:64), and the one IgG titer was 128.

Immediate allergic reactors. The reactions in these 12 patients consisted of generalized urticaria (6 patients), marked hypotension and urticaria (5 patients), and wheezing and rhinitis provoked by inhalation of penicillin powder in 1 patient. Reactions occurred between 3 and 20 minutes after contact with penicillin. Reactions were provoked by an intramuscular injection of procaine penicillin (7 patients), an oral penicillin tablet (1 patient), airborne penicillin powder (1 patient), and an intradermal skin test with 50 U of penicillin (3 patients). Table II shows the antibody assays performed 7 to 14 days after the reaction. In marked contrast to the control groups (Table I), all 12 immediate reactors had skin-sensitizing antibodies of minor determinant specificities (see Addendum). Five of these patients also had BPO-specific skin-sensitizing antibodies, and 58% of the group had IgG antibodies. The titers of IgM and IgG antibodies were not significantly different from the titers of the control groups (Figure 1, A and B). Serum assays for skin-sensitizing antibodies were done by passive transfer in 8 patients. Six of the 8 had detectable circulating skin-sensitizing antibodies of the same specificities as were detected by direct skin test (Table III). BPO-specific skin-sensitizing antibodies were not found in the sera of the patients with negative skin tests to BPO-polylysine. Serial assays beginning within 24 hours after the allergic reaction were done in 5 patients. In all 5, positive skin tests were obtained within 24 hours after the reaction, i.e., no period of "desensitization" was observed (Table III). Also, there was no change in the antibody pattern during the 14-day period after the allergic reaction, i.e., there was no "evolution" of the immune response. This observed absence of desensitization and evolution of the immune response may be related to the comparatively small antigenic stimuli received by these patients; they were given small amounts of penicillin (Table III). By contrast, many of the accelerated and late allergic reactors to penicillin showed a distinct evolution of the immune response (see below and Table III).

Accelerated allergic reactors. All 10 patients had generalized urticaria starting between 2 and 36 hours after the initiation of penicillin therapy. Urticaria persisted for 1 to 5 days. Antibody assays performed 7 to 14 days after penicillin therapy was discontinued are shown in Table II. In

TABLE II

Antibody assays in patients with recent allergic reactions to penicillin

Antibody pattern detected*	Percentage of patients having antibody pattern				
	Group 5, 12 patients with immediate allergic reactions†	Group 6, 10 patients with accelerated allergic reactions†	Group 7, 11 patients with late urticarial reactions†	Group 8, 16 patients with recurrent urticaria and arthralgia	Group 9, 19 patients with maculopapular and erythematous eruptions†
IgM alone (1:4 to 1:1,000)	0	0	0	0	58
IgM in high titers (1:2,000 to 1:16,000)	0	0	0	0	32
IgG	0	0	0	0	0
SSA(BPO) plus IgG plus IgM	0	60	91	0	5
SSA(minor) plus IgM	42	0	0	56	0
SSA(minor) plus IgG plus IgM	16	0	0	25	0
SSA(minor) plus SSA(BPO) plus IgG plus IgM	42	40	9	19	5

* IgM and IgG antibodies are BPO specific.
† Assayed 7 to 14 days after penicillin therapy was discontinued.

TABLE III

*Clinical histories and sequential antibody assays in selected patients**

Patient, age, sex	Clinical history	Days after: PG discontinued	Days after: Onset of reaction	Immunological tests: Direct skin tests for SSA: BPO-PLL	KPG	NaBPO	Passive transfer tests for SSA: BPO-PLL	KPG	NaBPO	HA titers†
				Immediate allergic reactors						
S.C. 39, M	Hypotension, urticaria 10 minutes after im PG	1	1	N	2+	2+				128/0
		13	13	N	3+	3+	N	2+	2+	128/0
		38	38	N	3+	3+				128/0
		180	180	N	3+	3+	N	1+	N	
B.K. 55, M	Hypotension, generalized pruritis 10 minutes after im PG	1	1	N	1+					4,000/4,000
		4	4	N	2+	2+	N	3+	3+	8,000/8,000
		8	8	N	2+	2+				
		25	25	N	2+	2+	N	1+	1+	8,000/8,000
E.A. 35, F	Hypotension, 5 minutes after oral PG	1	1	N			N	1+	3+	512/0
		4	4	N	1+	1+				
		10	10	N	2+	2+				
		120	120	N	2+	2+	N	1+	3+	256/0
J.B. 45, M	Generalized urticaria, 5 minutes after 50 U PG id	1	1	N	2+	N	N	N	N	256/64
		12	12	N	2+	N	N	1+	N	256/64
		35	35	N	2+	N				
E.S. 54, M	Lightheadedness, urticaria 5 minutes after 50 U PG id	Time of reaction		N	2+					
		7	7	N	2+		N	N	N	4,096/4,096
				Accelerated reactors						
L.M. 50, F	15 million U PG per day iv for 2 days; urticaria started 2 days after PG began and lasted 6 days	5	5	1+	N	1+	2+	N	N	800/200
		14	14	2+	N	2+				8,000/8,000
J.O. 24, M	1.8 million U procaine PG for 24 hours when urticaria started and lasted 1 day	4	4	1+	N	N				32/4
		7	7							32/16
		12	12	3+	N	N	3+	N	N	640/320
O.T. 55, M	1.8 million U procaine PG for 24 hours when urticaria began and lasted 1 day		1	3+	N	N	2+	N	2+	1,200/400
			9	3+	N	2+	4+	N	Trace	64,000/64,000
J.B. 56, M	20 million U PG per day for 6 weeks; urticaria started 24 hours after PG started and lasted 5 days		1	2+	N	N	2+	N	N	64/32
			8	1+	N	N				4,096/4,096
				Late urticarial reactors						
A.C. 34, M	1.8 million U procaine PG per day for 21 days; urticaria started on day 7 and lasted 1 day		2	N	N	N	2+	N	N	256/256
			8	3+	N	N				4,096/4,096
B.D. 63, F	1.2 million U procaine PG per day for 10 days when urticaria developed; it lasted 2 days	2	2	2+	N	N	3+	N	N	16,000/16,000
		3	3	2+	N	N				64,000/64,000
		9	9	2+	N	N	3+	N	N	64,000/64,000
				Maculopapular erythema reactors						
C.M. 70, F	1.2 million U procaine PG im for 10 days; eighth-day generalized maculopapular eruption faded in 6 days with scaling	1	3	N	N	N	N	N	N	256/32
		5	7	N	N	N	1+	N	N	8,192/512
		11	13	2+	N	N	2+	N	N	16,384/4,096
		14	16	2+	N	N	2+	N	N	8,192/2,046
J.R. 57, M	1.2 million U procaine PG im for 6 days, followed by 20 million U iv per day for 14 days; maculopapular eruption on day 10 of dosage that continued while PG was administered and faded with scaling 10 days after PG was stopped	4	14	N	N	N				1,024/0
		7	17	N	N	N				2,048/0
		12	22	N	N	N	N	N	N	2,048/0
		47	57	N	N	N				
L.D. 62, M	1.2 million U procaine PG per day im for 23 days; diffuse erythema and edema on day 23 clearing in 5 days	1	1	N	N	N				64/0
		3	3	N	N	N				512/0
		8	8	N	N	N				2,048/0
		15	15	N	N	N				2,048/0
F.S. 41, M	10 million U PG per day iv for 15 days; maculopapular eruption started on day 8, continued during PG administration, cleared 4 days after PG was stopped		2	N	N	N				256/0
		1	8	N	N	N				2,048/0
		7	14	N	N	N	N	N	N	2,048/0
		20	27	N	N	N				
Z.B. 65, M	2.4 million U PG im for 58 days; maculopapular eruption on lower extremities cleared in 7 days after PG was stopped	3	3	2+	N	N				320/128
		10	10	2+	N	N				1,600/256
B.H. 79, M	2.4 million U procaine PG per day for 13 days when diffuse maculopapular eruption started; cleared in 2 days after PG was stopped	2	2	N	N	N				1,600/100
		7	7	1+	N	N	2+	N	N	6,400/1,600

* Abbreviations: id, intradermally; N, negative; BPO-PLL, BPO-polylysine; KPG, penicillin; NaBPO, sodium benzylpenicilloate; HA, hemagglutination.

† See Methods for expression of HA titers.

contrast to the controls (Table I), all 10 accelerated reactors had BPO-specific skin-sensitizing antibodies, which in all cases were associated with IgG and IgM antibodies. Titers of IgG antibodies were significantly higher in this group than in the control groups (compare sections A, B, and C of Figure 1). The geometric mean titer was 1:8,192 for IgG. Four patients had skin-sensitizing antibodies of minor determinant specificities in addition to BPO-specific skin-sensitizing antibodies, IgG, and IgM antibodies.

Serial antibody assays were done in 4 patients. All 4 showed an evolution of the immune response. This consisted of a marked rise in IgG titers, and in several patients an increase in intensity of BPO-specific skin tests (Table III). In 3 patients, initial skin tests were done within 24 hours after the onset of the allergic reaction. All 3 gave positive skin tests to BPO-polylysine when first tested. Thus, desensitization of skin reactivity after accelerated reactions was not observed in our patients. In 2 patients, however, the intensity of the BPO-specific skin test increased during the next 7 days (Table III). This finding may reflect the evolution of the immune response rather than desensitization. In 2 patients (patients J.B., Table III) penicillin therapy was continued despite the accelerated urticarial reaction. Urticaria ceased spontaneously after 3 and 5 days, respectively. This spontaneous cessation of urticaria correlated temporally with a marked rise in IgG titer; skin tests to BPO-polylysine remained positive.

Late urticarial reactors. This group consisted of 11 patients who developed generalized urticaria after 8 to 21 days of penicillin therapy. In all, urticaria cleared spontaneously in 1 to 5 days. Antibody assays performed 7 to 14 days after the onset of the rash are shown in Table II. All 11 late reactors had BPO-specific skin-sensitizing antibodies and IgG and IgM antibodies. IgG titers were significantly higher than those of the control groups (Figure 1, B, C). The mean geometric titer for IgG was 1:8,192. One patient had skin-sensitizing antibodies of minor determinant specificities in addition to BPO-specific skin-sensitizing antibodies and IgG and IgM antibodies. Thus, the immune responses of late urticarial reactors and accelerated urticarial reactors were essentially the same (Table II). Serial studies were done in 2 patients (Table III). Both showed evolution of the immune response during the 14-day period after onset of the reaction. In 1 late reactor, penicillin therapy was continued despite the urticarial reaction. Urticaria cleared spontaneously in 24 hours coincident with a marked rise in IgG titer; the BPO-polylysine skin test remained positive. Similar observations were made in 2 accelerated reactors (see above).

Recurrent urticaria and arthralgia. This group consisted of 16 patients who presented a clinical syndrome of three unusual features: *1*) All had recurring episodes of generalized urticaria (5 had angioedema of lips and extremities, in addition to urticaria) that recurred for 2 to 15 weeks after penicillin therapy had been discontinued. In 10, urticaria ceased spontaneously; in 6, urticaria ceased coincident with a diet free of milk and milk products [some milk samples contain penicillin (19), and probably also degradation products of penicillin].

2) Thirteen of the 16 patients complained of stiffness and pain on motion of the joints. Symmetrical involvement occurred in all cases; the fingers, wrists, toes, ankles, and knees were most frequently involved; arthralgias recurred coincident with urticaria. Physical examination showed edema and erythema of the skin surrounding the joints. It was not clear whether the joints themselves were involved. There was no clear evidence of fever, lymphadenopathy, or cardiac or renal involvement associated with these reactions.

3) These reactions occurred after oral or parenteral therapy. In 8 patients, they began between 3 and 21 days after penicillin therapy had been discontinued. By contrast, immediate, accelerated, late urticarial, and maculopapular reactions began while the patient was still being treated with penicillin, or very shortly after.

Antibody assays were done between 1 and 4 weeks (14 patients) and 5 and 12 weeks (2 patients) after the onset of the allergic reaction in this group, since the patients were seen initially at three times. Results are given in Table II. In marked contrast to the control groups, all 16 patients had skin-sensitizing antibodies of minor determinant specificities. Three patients also had BPO-specific skin-sensitizing antibodies. The IgG and IgM titers in these patients did not differ from those of the control groups. The immune

TABLE IV

Specificity of skin tests in immediate and recurrent urticarial reactors*

Patients	Number of patients with positive skin tests		
	Only NaBPO	Only KPG	Both NaBPO and KPG
Immediate reactors (12 patients)	2 (17%)	4 (33%)	6 (50%)
Recurrent urticarial reactors (16 patients)	6 (38%)	0	10 (62%)

* Wheal and flare reactions. NaBPO and KPG both at 1×10^{-2} molar concentration.

responses of these patients are thus similar to the immune responses of the immediate allergic reactors (Table II). However, immediate allergic reactors tended to give positive immediate skin tests to penicillin more frequently, whereas recurrent urticarial reactors tended to give positive skin tests more frequently to sodium benzylpenicilloate (Table IV), indicating different minor determinant specificities of the skin-sensitizing antibodies associated with these allergic reactions. Sequential antibody assays initiated after the tenth day following discontinuation of penicillin therapy showed no increase in IgM or IgG titers and no appearance of new skin-sensitizing antibodies.

Maculopapular and diffuse erythema reactions. This group consisted of 19 patients. Seventeen developed maculopapular eruptions of the trunk and proximal extremities; 2 had confluent areas of erythema. In all, the rashes developed while the patients were still on penicillin therapy and after 3 to 58 days (most frequently, 6 to 11) of parenteral penicillin therapy. The eruptions cleared with some scaling between 3 to 21 days after the drug was discontinued. There was no clear evidence of fever, lymphadenopathy, or renal or cardiac involvement associated with these reactions. None of these patients had arthralgias.

Antibody assays performed between 7 and 14 days after penicillin therapy was discontinued are shown in Table II. Six of the 19 patients developed unusually high titers of IgM antibodies (1:2,000 to 1:16,000). By contrast, the geometric mean IgM titer for the 103 patients in the control group (Table I, group 3) was 1:64, and none had IgM titers above 1:1,024 (Figure 1, B). Sequential antibody assays were done in 3 of the 6 patients (days 1 to 3 and again between days 7 to 12). They showed a rise in IgM titer of 3, 4, and 5 tubes (8- to 32-fold) (Table III). In contrast, sequential IgM assays over the same time period done on 10 consecutive controls showed rises in titer of 1 to 2 tubes (two- to fourfold). These 6 patients showed a striking evolution of the immune response (Table III). Three of the 6 had BPO-specific skin-sensitizing antibodies and IgG antibodies when assayed at 7 to 14 days. However, in 2 of these 3 patients, skin-sensitizing antibodies appeared after the allergic reaction had cleared, in contrast to the immediate, accelerated, and late reactions where skin-sensitizing antibodies were detected immediately after the onset of the allergic reaction (see above). Of the 13 remaining patients, 4 had borderline high IgM titers of 1:1,024 and, accordingly, may belong with the high IgM group. Two more patients had IgM antibodies in high titers (1:16,000 and 1:128,000) and BPO-specific skin-sensitizing antibodies when assayed 12 and 14 days after penicillin therapy was discontinued. Both patients also had positive delayed hypersensitivity skin tests to 0.1 M penicillin (20). These are distinctly unusual immune responses to penicillin. However, whether or not these two patients had a predominantly IgM response early could not be determined, since the first assays were done at 12 days (Table III). The remaining 7 patients in this group did not have an unusual immune response to penicillin. They had only low titers of IgM antibodies; the mean geometric titer for the 7 patients was 1:64, which is identical to the controls. Furthermore, delayed hypersensitivity skin tests with 0.1 M penicillin (20) were negative for all 7. Six of the 7 patients were treated with the procaine salt of penicillin, and all 7 were being treated with other allergenic drugs, e.g., aspirin, streptomycin, and barbiturates.

Association of antibodies. It was found previously (13) that 10 consecutive IgG sera (i.e., sera whose hemagglutination titers were not reduced by treatment with mercaptoethanol) contained comparatively low concentrations of BPO-specific IgM antibodies and antibodies of other unidentified antibody classes (probably including IgA). Accordingly, BPO-specific IgG antibodies appear to be commonly associated with lower titers of IgM antibodies. In this study, we found that all of the 47 patients who had BPO-specific skin-sensitizing antibodies also had BPO-specific IgG antibodies (Tables I, II). Furthermore, 15 of the 43

(34%) patients with BPO-specific skin-sensitizing antibodies also had skin-sensitizing antibodies of minor determinant specificities, and 15 of the 35 (43%) patients with skin-sensitizing antibodies of minor determinant specificities also had BPO-specific skin-sensitizing antibodies, when assayed at 7 to 14 days after penicillin therapy was discontinued (Tables I, II). In contrast, among the 103 patients in group 3 (Table I), only 8 of 102 (8%) patients who did not have skin-sensitizing antibodies of minor determinant specificities had BPO-specific skin-sensitizing antibodies, and none of the 94 patients who did not have BPO-specific skin-sensitizing antibodies had skin-sensitizing antibodies of minor determinant specificities. These results demonstrate a significant association of BPO-specific IgG with BPO-specific skin-sensitizing antibodies and of BPO-specific skin-sensitizing antibodies with skin-sensitizing antibodies of minor determinant specificities.

Relationship of atopic background and immune response to penicillin. Of the 171 patients in this study who had recently completed courses of penicillin therapy (Tables I and II, groups 3, 5–9), reasonably reliable personal and family histories of atopic allergy were obtained in 146. They included 82 of group 3, 11 of group 5, 10 of group 6, 10 of group 7, 16 of group 8, and 17 of group 9. Fifteen of the 146 were classified as atopic. Seven had histories of seasonal asthma or rhinitis, 6 had histories of perennial asthma starting before the age of 30, 1 had generalized urticaria to shrimp, and the other had an anaphylactic reaction to tetracycline [reported in (21)]. Fifteen were classified as "questionably atopic." Eight had histories of wheezing with respiratory infections, 6 had perennial asthma starting after the age of 30, and 1 had perennial rhinitis. The family history was considered positive for atopy when a parent, child, sibling, aunt, uncle, or first cousin had a history of seasonal or perennial rhinitis or asthma or urticaria provoked by specific foods.

TABLE V

Relation of atopic background to immune response to PG (synthesis of SSA)

Atopic background	Number of patients with detectable SSA 7 to 14 days after PG therapy			
	No SSA	SSA (BPO)	SSA (minor)	Both SSA(BPO) and SSA(minor)
Atopic (15 patients)	3	3	5	4
Questionably atopic (15 patients)	5	3	3	4
Nonatopic, but with family history of atopy (6 patients)	2	0	3	1
Nonatopic, and no family history of atopy (110 patients)	79	16	9	6
Totals:	89	22	20	15

TABLE VI

Relation of personal history of atopy to atopic family history

Personal past history of atopy	Fraction with family history of atopy
Atopic	3/15 (20%)
Questionably atopic	2/15 (13%)
Nonatopic	6/116 (5%)

Table V shows that 12 of the 15 (80%) atopics had skin-sensitizing antibodies and that 9 of the 15 (60%) had skin-sensitizing antibodies of minor determinant specificities. In contrast, 31 of 110 (28%) nonatopic subjects had skin-sensitizing antibodies, and 15 of the 110 (14%) had skin-sensitizing antibodies of minor determinant specificities. The fact that this group included many allergic reactors to penicillin does not appear to invalidate this observation, as the pertinent correlation is between synthesis of skin-sensitizing antibodies and atopy. Table VI shows that a significantly higher percentage of atopic than nonatopic patients had family histories of atopic allergic diseases.

Discussion

The foregoing results describe a heterogeneity of immune responses to penicillin in man. Heterogeneity was manifested by individual differences in haptenic specificities of antipenicillin antibodies synthesized, in the major classes of BPO-specific antibodies, and in the serum concentrations achieved.

Two factors influencing the individual immune response are the dosage (or route of administration) of penicillin and the atopic background of the patient. We found that twice as many patients synthesized BPO-specific IgG antibodies and skin-sensitizing antibodies among patients re-

cently treated with penicillin than among patients who had not had penicillin therapy for at least 2 years, whereas virtually all patients of both groups made IgM antibodies. Continued synthesis of small amounts of IgM antibodies in the latter group is tentatively ascribed to repeated contacts with trace amounts of penicillin present in environment (19). Treatment with large doses of penicillin given intravenously appeared to decrease the percentage of patients synthesizing IgG and skin-sensitizing antibodies. This observation requires verification with more patients. Its significance is not yet clear. It may represent induction of partial immune tolerance.

About a three- to fourfold higher percentage of atopic than nonatopic patients were found to synthesize skin-sensitizing antibodies, and the difference was somewhat more striking for skin-sensitizing antibodies of minor determinant specificities. This "atopic" factor appears to be genetically controlled, at least in part, since a family history of atopy occurred four times more frequently in atopic than in nonatopic patients. No significant effect of age, sex, or concurrent infection on the immune response was detected in this study.

A significant "linking" of the synthesis of various antipenicillin antibodies was observed. All patients recently treated with penicillin who synthesized BPO-specific skin-sensitizing antibodies also synthesized BPO-specific IgG antibodies. Furthermore, we observed a marked association of skin-sensitizing antibodies of minor determinant specificities and BPO-specific skin-sensitizing antibodies.[5] Whether this linked synthesis of skin-sensitizing antibodies of different specificities reflects only a constitutional ability to synthesize skin-sensitizing antibodies or whether environmental factors are also involved is unknown. Clinically, this observation may provide a basis for the widely held notion that patients with active atopic allergic diseases are more likely to develop atopic allergy to immunologically unrelated allergens.

Patients with allergic reactions to penicillin made a qualitatively and quantitatively greater immune response to penicillin than did the nonallergic reactor controls.

The immediate allergic reactors all had skin-sensitizing antibodies of minor determinant specificities (see Addendum); a significantly high percentage also had BPO-specific skin-sensitizing antibodies, whereas there was no striking difference from the controls in frequency or titer of IgG or IgM antibodies. These "association data" suggest that immediate allergic reaction to penicillin are most often mediated by skin-sensitizing antibodies of minor determinant specificities. BPO-specific skin-sensitizing antibodies were found to be invariably associated with accelerated and late urticarial reactions and probably mediate these reactions. We suspect that BPO-specific skin-sensitizing antibodies usually do not mediate immediate allergic reactions because of their virtually invariable association with BPO-specific IgG (and IgM) antibodies, which act as "blocking antibodies." Theoretically, immediate allergic reactions due to BPO-specific skin-sensitizing antibodies might occur even in the presence of BPO-specific blocking antibodies, if the avidity for antigen were much greater for the skin-sensitizing antibodies. However, in general clinical practice, this usually does not occur.

That BPO-specific IgG antibodies can compete for antigen with BPO-specific skin-sensitizing antibodies and thus slow or prevent release of vasoactive amines (i.e., act as blocking antibodies) is suggested by the present retrospective clinical study and by prospective studies (17, 18). Thus, accelerated and late urticarial allergic reactions associated with BPO-specific skin-sensitizing antibodies were found to cease spontaneously, coincident with the sharp rise in the titer of BPO-specific IgG antibodies (representing a secondary immune response to penicillin) despite the persistence of BPO-specific skin-sensitizing antibodies and the continued administration of penicillin. Prospectively (17, 18), none of 6 patients with BPO-specific IgG and skin-sensitizing antibodies who were treated with intravenously administered penicillin developed immediate allergic reactions. However, in 4 of these 6 patients, the titers of IgG antibodies dropped (representing absorption of antibodies by BPO conjugates formed from penicillin *in vivo*) during the first 18 to 72 hours of penicillin therapy. These 4 patients developed urticaria coinciding with the low titers of IgG antibodies; urticaria ceased abruptly when the IgG antibody titers rose. The other two patients maintained high titers of IgG antibodies and did not develop allergic reactions (17, 18). Thus,

the familiar clinical observation that accelerated and late urticarial reactions to penicillin frequently clear spontaneously as penicillin therapy is continued appears to be due to a rise in concentration (and possibly in binding affinity) of BPO-specific IgG antibodies, and not to desensitization or absorption of skin-sensitizing antibodies. Final proof for the role of BPO-specific antibodies as blocking antibodies against BPO-specific skin-sensitizing antibodies will require studies utilizing purified antibodies and model systems *in vitro* and *in vivo*.

Implied in the suggestion that immediate reactions to penicillin are mediated mainly by skin-sensitizing antibodies of minor determinant specificities is the view that skin-sensitizing antibodies of minor determinant specificities are generally not associated with high serum activities of blocking antibodies of comparatively high binding avidities of the same haptenic specificities. Proof of this view will require the development of assay methods for IgG and IgM antibodies of the minor determinant specificities.

The syndrome of recurrent urticaria and arthralgia described in Results was found to be invariably associated with skin-sensitizing antibodies of minor determinant specificities. On the basis of this striking correlation, the urticarial element of this syndrome appears to probably be mediated by skin-sensitizing antibodies of minor determinant specificities. The haptenic specificities of the minor determinant generally associated with this syndrome and with immediate allergic reactions appear to differ, as described in Results. The recurrent nature of this syndrome (as contrasted with accelerated and late urticarial reactions) may be ascribed tentatively to the possibility mentioned above that skin-sensitizing antibodies of minor determinant specificities are less likely to be associated with significant activities of blocking antibodies than are BPO-specific skin-sensitizing antibodies. Accordingly, small amounts of antigen derived from penicillin and its degradation products present in the environment could be sufficient to initiate release of vasoactive amines. Whether "arthralgia" in this syndrome is due to true joint involvement or to extensive edema involving the periarticular tissues is not known. If true arthritis does occur, its immunological mechanism is obscure.

Six or 10 of the 17 maculopapular reactors developed unique BPO-specific IgM antibody responses to penicillin, i.e., relatively low serum titers just after penicillin was discontinued, rising to unusually high titers in 5 to 12 days. This kind of pattern is seen for 7 S antibodies in experimental serum sickness in rabbits (22). Accordingly, this observation constitutes initial evidence that the maculopapular reactions in the 6 or 10 patients might have been mediated by soluble toxic antigen-antibody complexes of BPO-specific IgM antibodies. Tada and Ishizaka have recently shown toxicity of such complexes (of rabbit IgM) in the skin of rabbits (23). However, proof of this view will require demonstration of such complexes in the serum during the first days of the reaction, and in the skin lesions.

Seven of the 19 maculopapular reactors had immune responses to penicillin indistinguishable from those of control subjects. Either these patients had allergic reactions to other simultaneously administered drugs (e.g., procaine, barbiturates, streptomycin), or these reactions may have been due to penicillin antibodies that are not yet detectable (e.g., IgG or IgM of minor haptenic specificity), or not all maculopapular reactions are allergic in mechanism, or unknown factors may be operating. As to the last possibility, the serum concentration of BPO conjugates (formed from penicillin) may be important. Suitable assay methods for serum BPO conjugates are not yet available.

Work on the delayed hypersensitivity immune response to penicillin is in progress (20). Studies on the minor determinant-specific immune responses to penicillin would be of importance, especially pertaining to the immunological mechanisms of immediate and recurrent urticarial allergic reactions. Presently, these studies are being prevented by insufficient knowledge of the chemistry of the minor haptenic determinants.

Summary

The immune responses of man to benzylpenicillin and the immune mechanisms of penicillin allergy were studied by serial antibody assays of various groups of patients for benzylpenicilloyl-specific skin-sensitizing antibodies, IgG and IgM antibodies, and skin-sensitizing antibodies of minor determinant specificities.

Antipenicillin antibodies were detected in virtually all patients studied, including those who denied ever having penicillin therapy. Recent penicillin therapy increased the frequency of IgG and benzylpenicilloyl-specific skin-sensitizing antibodies. Three to four times as many atopic as nonatopic patients responded to penicillin therapy by synthesizing skin-sensitizing antibodies. Virtually all patients with benzylpenicilloyl-specific skin-sensitizing antibodies also made benzylpenicilloyl-specific IgG antibodies. The synthesis of benzylpenicilloyl-specific skin-sensitizing antibodies and skin-sensitizing antibodies of the minor haptenic determinants (apparently distinct antibodies) was "linked"; a patient who synthesized one was five to ten times more likely to synthesize the other than were the control subjects.

Allergic reactors made qualitatively and quantitatively more intense immune responses to penicillin than did nonreactors. Clinical and immunological correlations suggest that immediate allergic reactions are mediated by skin-sensitizing antibodies usually of minor determinant specificities, accelerated and late urticarial reactions are mediated by benzylpenicilloyl-specific skin-sensitizing antibodies, the recurrent urticaria-arthralgia syndrome is mediated by skin-sensitizing antibodies of minor determinant specificities, and some maculopapular erythema reactions may be mediated by soluble toxic antigen-antibody complexes of benzylpenicilloyl-specific IgM antibodies. Accelerated and late urticarial reactions to penicillin appear to terminate spontaneosuly by the action of benzylpenicilloyl-specific IgG antibodies, which act as "blocking antibodies" in competition with benzylpenicilloyl-specific skin-sensitizing antibodies.

Addendum

Since submission of this manuscript, we have observed 2 patients with recent immediate allergic reactions to penicillin who had BPO-specific skin-sensitizing antibodies and no detectable minor determinant specific skin-sensitizing antibodies when tested 2 and 7 days after the reaction. Both patients had relatively low titers of BPO-specific IgG antibodies (1:200) and evidence of relatively high-avidity skin-sensitizing antibodies. During the same period, 4 additional immediate allergic reactors with skin-sensitizing antibodies of minor determinant specificities were observed.

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