

From the Finsen Institute, Medical Department (Head: Nis I. Nissen, M.D.) and Rigshospitalet (the University Hospital), Medical Out-patient Clinic (Head: J. Hess Thaysen, M.D.), Division for Allergic Diseases (Head: Egon Bruun, M.D.), Copenhagen, Denmark.

SKIN-SENSITIZING ANTIBODIES IN
SERUM FROM PATIENTS WITH PENICILLIN
ALLERGY STUDIED BY PASSIVE
TRANSFER TO MONKEY SKIN AND
COMPARED WITH RESULTS OBTAINED
ON HUMAN SKIN
(PRAUSNITZ-KÜSTNER TECHNIQUE)

By

JENS PEDERSEN-BJERGAARD

INTRODUCTION

Reagins—skin-sensitizing antibodies—were first demonstrated in the serum of patients with allergic diseases of the immediate type by *Prausnitz & Küstner* (1921) and *de Besche* (1922). Following passive transfer by intracutaneous injection of serum from an allergic person to a non-allergic recipient, a positive cutaneous reaction could be elicited in the recipient by the injection of specific antigen into the sensitized site.

Since the first demonstration of the reagins their structure has been subject to numerous investigations. It has been established that they are immune globulins of the γ -E group (*Ishizaka, Ishizaka & Hornbrook* 1966) of a structure like other immune globulins (*Ishizaka, Ishizaka & Terry* 1967) having a molecular weight of 196,000 and skin attachment localized to the Fc fragment (*Stanworth, Humphrey, Bennich & Johansson* 1968).

Reagins play a central pathogenetic role in allergic diseases

of the immediate type. This is evident int. al. from the fact that they can transmit the disease by intravenous infusion of serum from an allergic donor to a non-allergic recipient. For instance, passive sensitization to horse dander has been demonstrated following blood transfusion from a donor with horse allergy; after the sensitization the recipient developed typical attacks of asthma when exposed to horses (*Ramirez 1919*).

Determination of reagins is of great practical importance in allergic diseases of the immediate type. Frequently, it is possible to assess the significance of positive cutaneous reactions with numerous antigen extracts in allergic patients, as their serum usually contains only reagins with specificity for one or a few relevant antigens (*de Besche 1929*). The reagin titre of allergic patients' serum can, moreover, in most cases be correlated with the cutaneous reactivity to the same antigen (*Connell & Sherman 1964 a*), and finally reagin determination is applicable as a measure of clinical sensibility (*Levine & Coca 1926*, *Delorme, Richter, Grant, Blumer, Lenzhoff & Rose 1961*, *Connell & Sherman 1964 b*).

In *penicillin allergy* determination of reagin is of quite particular diagnostic interest. Cutaneous testing of penicillin allergics with penicillin, even though performed with the utmost caution, may occasionally give rise to serious systemic reaction. For instance, systemic reaction has been observed after intracutaneous test using benzylpenicillin 5×10^{-6} units (*Coleman & Swineford 1956*), 1×10^{-5} units, 1×10^{-4} units, and 2×10^{-3} units (*Pedersen-Bjergaard 1967*). Similarly, intracutaneous tests using penicilloyl-polylysine may entail systemic allergic reaction (*Resnik & Shelley 1966*). However, a good correlation between skin sensitizing antibodies and positive cutaneous reactions (immediate type) to penicillin, penicilloyl, and penicilloic acid (Fig. 1) has been demonstrated in penicillin allergics (*Redmond & Levine 1967*), especially when the patient is examined less than 2 months after the allergic reaction took place. Thus, reagin determination ought to be able to replace to a great extent the risky cutaneous testing of penicillin allergics.

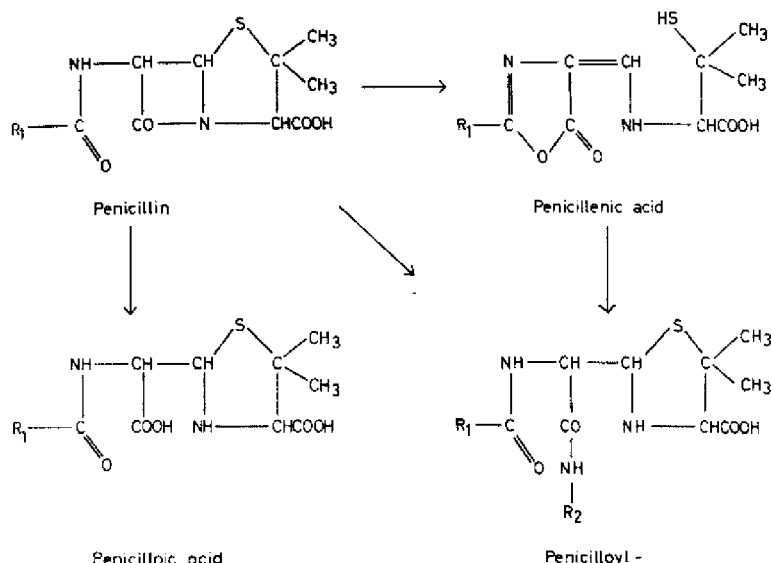


Fig. 1.

Reagin determination using the Prausnitz-Küstner technique has not been used as widely as might have been expected for routine diagnostic purposes in allergy. There are several reasons: The investigation requires human recipients, it is time-consuming, and the quantitation is cumbersome. Serum transfer of infectious diseases to the recipient, in particular hepatitis, may occur, and in addition the recipient may become sensitized by or be allergic to the serum samples or antigens.

Therefore, passive transfer of human reagins to various animal species has been tried. Positive results, with local sensitization of skin following intracutaneous injection of serum from allergics have been obtained in various *lower species of monkeys* which may also be sensitized systemically by intravenous injection of serum from allergic patients (Grove 1928). The reactions must be attributed to reagins— γ -E globulin—(Ishizaka, Ishizaka & Arbesman 1967), but monkey skin seems to be somewhat less susceptible than human skin to sensitization by reagins. Thus, the reagin titre was

found to be about 10 times higher on passive transfer to human skin than on transfer to monkey skin (*Patterson, Fink, Nishimura & Pruzansky* 1965) in studies using ragweed and horse serum antigen.

Guinea-pig and rat skin has also been sensitized by serum from allergics. However, the antibodies which cause passive sensitization of these animals do not appear to be reagins, but γ -G globulins, and the results have accordingly often shown correlation to titre values in agglutination reactions (*Rose, Kent, Reisman & Arbesman* 1964, *Ovary* 1964, *Levine, Fellner, Levytska, Franklin & Alisberg* 1966). Moreover, the results of passive transfer to rat skin have been extremely inconstant (*Sonntag & Marcus* 1963).

Demonstration of reagin by passive transfer to monkey skin has previously been reported with serum from a patient with penicillin allergy (*Layton, Yamanaka, Greene & Perlman* 1963). A positive result, with demonstration of reagin, was obtained on monkeys of the species *Macca-rhesus*, *Macca-irus*, *Macca-speciosus*, and *Cebus capucinus*; the reaction was elicited by penicillin. In the serum of penicillin allergics, however, the Prausnitz-Küstner technique may show not only reactions elicited by *penicillin* (*Criep* 1944, *Matheson & Elegant* 1955, *Ljaljevic* 1959), but also reactions to the *penicilloyl radical* (*van Arsdell, Tobe & Pasnick* 1963, *Siegel & Levine* 1964), and to *penicilloate* the salt of penicilloic acid (Fig. 1) (*Redmond & Levine* 1967).

The *object* of the present study, therefore, was to assess reagin determination by passive transfer to monkey skin in a fairly large serological material with a view to the applicability of this method in the practical diagnosis of penicillin allergy, to perform the studies with penicillin, penicilloyl compounds as well as penicilloate, and to compare the results with quantitative reagin determination using the Prausnitz-Küstner technique.

MATERIALS AND METHODS

Serum from 53 patients with proven penicillin allergy was studied. In 48 the diagnosis had been confirmed by provocation tests with penicillin and in 5 by a definite and recent history of penicillin allergy or by positive cutaneous reactions (Pedersen-Bjergaard, to be published). All sera were removed and stored sterile at -20°C until the investigations could be carried out. Immediately before use the samples were thawed and used direct for a Prausnitz-Küstner reaction or for passive transfer tests on monkey skin. Sterile filtration of the samples was not done, as such a procedure may weaken the reagin activity, especially if the serum is not used until some time after the sterile filtration (Sehon, Fyles & Rose 1955).

Passive transfer tests were performed on 40 monkeys of the species *Macaca-irus* (*cynomolgus*), weight 1.9–5.7 kg, average 3.4 kg. The majority were youngish animals not previously treated with penicillin, human serum, or human tissue. Prior to the investigations the monkeys were anaesthetized by intramuscular injection of Nembutal® 6 per cent $\frac{1}{2}$ –1 ml/kg. After the anterior surface of the chest and abdomen had been shaved, 0.05 ml serum from various penicillin allergies was injected intracutaneously into a number of marked fields.

48 hours later the monkeys were again anaesthetized, and through a scalp vein needle 2 ml/kg body weight of a 0.5 per cent solution of Evans Blue (T 1824) was injected into the femoral vein to visualize positive reactions (Layton, Lee & Yamanaka 1962). Thereafter, the various antigen solutions were injected intravenously in alternating succession at intervals of 10–15 min. Positive reactions were distinct blue patches, at least 5 mm in diameter, appearing in the sensitized sites after the injection of antigen (Fig. 2).

Prausnitz-Küstner reaction was done on 26 human recipients between 24 and 54 years of age. All the recipients were in good general condition and were not at the time of the study receiving any treatment that might influence the results. The recipients were sensitized by intracutaneous injections of 0.1 ml serum from penicillin allergies into a number of pre-marked fields on the back. Reactions were elicited at the end of 24 hours by injecting antigen into the recipient, either intravenously or intracutaneously into the sensitized sites. Positive reactions were typical wheals, at least 5 mm in diameter and surrounded by 10 mm erythema, all differing clearly from a control reaction on non-sensitized skin.

The recipients had no history of penicillin allergy, and immediately prior to each provocation of Prausnitz-Küstner reaction by antigen injection an intracutaneous test was performed by 0.05 ml antigen solution on the forearm, far from the sensitized area on the recipient's back.

The Prausnitz-Küstner reaction as well as passive transfer to monkey skin was performed with each individual sample of serum at least twice on satisfactorily reacting recipients. With all sera, an attempt was made to provoke the reactions by preparations of benzylpenicillin, penicilloyl-polylysine as well as benzylpenicilloate in alternating succession.

Positive reference sera were used in all experiments.



Fig. 2.

Macca-irus monkey showing satisfactory reactions after passive transfer of reaginic serum from penicillin allergies followed by intravenous injection of Evans Blue, Benzylpenicillin and Penicilloyl-Polylysine.

Reagents

Danish commercial crystalline benzylpenicillin in the form of the sodium salt (Penicillin Novo® and Penicillin Leo®).

Sephadex purified benzylpenicillin in the form of the sodium salt (lot 681022 and 671121) freeze-dried immediately after the purification (Leo Pharmaceutical Products Trading Ltd., Copenhagen).

Penicilloyl-Polylysine Sigma "Cilligen"® PPL 1 containing 1×10^{-5} M and PPL 50 containing 50×10^{-5} M penicilloyl compounds and less than 0.05 units penicillin/1 ml.

Sodium benzylpenicilloate (preparation 17295 G) made of Sephadex-purified penicillin by hydrolysis with NaOH at pH 11 (titrator) for 24–36 hours. Penicilloate concentration 1.7×10^{-1} M. The preparation contained less than 0.05 units penicillin/10 ml (Leo Pharmaceutical Products Trading Ltd., Copenhagen).

Evans Blue (T 1824) 0.5 per cent aqueous solution USP Warner-Chilcott.

Injectable histamine chloride 0.01 per cent (University Hospital Dispensary, Copenhagen).

RESULTS

On passive transfer to monkey skin reagins were demonstrated in 10 out of 53 sera from penicillin allergies.

Sodium benzylpenicillin, 10,000 units/kg body weight, administered intravenously to the monkeys, elicited positive reactions in 6 sera (Table 1). No demonstrable difference between the results obtained with commercial and with Sephadex-purified penicillin.

Penicilloyl-Polylysine "Cilligen" PPL 1 (1×10^{-5} M) 1.5 ml administered intravenously to the monkeys elicited positive reactions in 8 sera (Table 1).

Sodium benzylpenicilloate (1.7×10^{-1} M) 2.0 ml/kg body weight administered intravenously to the monkeys elicited positive reactions in 7 sera (Table 1).

In 3 sera, passive transfer to monkey skin showed a reaction only to penicillin, in 5 only to penicilloyl-polylysine, and in 3 only to penicilloate. In 1 serum there were reactions to penicillin as well as to penicilloyl, in 2 to penicillin and penicilloate, and in 2 to penicilloyl and penicilloate.

In 18 monkeys sensitization by passive transfer was a complete failure, no reactions being obtained, even with sera which had shown a high reagin titre in Prausnitz-Küstner ex-

TABLE 1

Passive Transfer to Monkey Skin and to Human Skin (Prausnitz-Küstner's Technique) of Sera from 53 Patients with Penicillin Allergy. Reactions Elicited by Benzylpenicillin, Penicilloyl-Polysine and Benzylpenicilloate.

Serum sample	Reaction on monkey skin			Titre on human skin		
	peni- cillin	peni- cilloyl—	peni- cilloate	peni- cillin	peni- cilloyl—	peni- cilloate
EMW	+	—	+	1:1000	1:10	1:100
ELP	+	—	—	1:10	—	—
OMN	—	—	—	—	—	—
EKS	+	—	+	1:100	—	1:10
EJ 1	—	—	—	1:10	—	—
MHJ	+	—	—	1:1	1:1	—
EMT	—	+	—	—	1:10	—
ELC	—	—	—	—	—	—
HHM	—	—	—	—	—	—
KU	—	+	+	—	1:1000	1:10
EJ 2	—	+	—	—	1:100	—
AEL	—	—	—	—	—	—
KKT	—	—	—	—	—	—
JVN	—	—	—	1:10	—	—
MAS	—	—	+	1:10	—	1:100
OEP	—	+	—	1:10	1:100	1:1
OCC	—	—	+	1:1	1:1	1:10
EVP	—	—	—	—	1:1	—
EGC	—	—	—	—	1:1	1:10
DJ	+	+	—	1:10	1:100	1:10
EJN	—	+	+	—	1:1000	1:100
EMB	—	—	—	—	—	—
AMJ	—	—	—	—	—	—
ATJ	—	—	—	—	—	—
MH	—	—	—	—	—	—
AK	—	—	—	—	—	—
ANT	—	—	—	—	—	—
MOP	—	—	—	1:1	—	—
HEN	—	—	—	—	1:1	—
IV	+	—	—	1:10	1:1	—
KÄH	—	—	+	—	1:10	1:1
AGJ	—	—	—	—	—	—
FRB	—	—	—	—	—	—
TP	—	—	—	—	—	—
JPP	—	—	—	—	—	—

TABLE 1 (cont.)

Serum sample	Reaction on monkey skin			Titre on human skin		
	peni-cillin	peni-cilloyl -	peni-cilloate	peni-cillin	peni-cilloyl -	peni-cilloate
JMW	—	—	—	—	—	—
HP	—	+	—	—	1 : 10	—
JEM	—	—	—	—	—	—
EC	—	—	—	—	1 : 1	—
JS	—	—	—	1 : 1	—	—
MR	—	—	—	—	—	—
KED	—	—	—	—	—	—
MPA	—	—	—	—	—	—
ASJ	—	—	—	—	—	—
KLH	—	—	—	1 : 1	—	—
ALJ	—	+	—	—	1 : 100	—
HMP	—	—	—	—	1 : 10	1 : 1
EF	—	—	—	—	1 : 1	—
OBO	—	—	—	—	—	—
JJN	—	—	—	—	—	—
OMJ	—	—	—	—	—	—
BSJ	—	—	—	1 : 1	—	—
EJ 3	—	—	—	—	1 : 1	1 : 1

— negative reaction. + positive reaction. 1 : 1 titre of positive reaction.

periments. In 8 monkeys the reactions were on the whole weak and positive only for the best reacting sera. Only 14 monkeys were satisfactorily sensitized (Fig. 2), but even in these 14 animals the susceptibility appeared to vary. The results were somewhat unreliable (Table 2), in particular with sera having reagin titres of 1 : 10 and less.

The ability for sensitization appeared to be independent of the monkey's sex or weight. 9 monkeys were subjected to intracutaneous tests using injections of histamine chloride 0.01 per cent. A highly positive reaction was obtained in 3 out of 4 monkeys satisfactorily sensitized by human reagins. 3 monkeys that could not be sensitized showed strong, weak, and no reaction respectively to injections of histamine. The poor or lacking sensitization of numerous monkeys, like the weak and

negative reactions to the histamine injection, was not in any case due to errors in the i. v. injection of T 1824 and antigen. According to the investigations the number of transfer tests to each monkey and a shorter interval between sensitization and provocation of reactions had no influence upon the attainment of satisfactory results. Infection in the sensitized cutaneous areas did not occur.

After sensitization and provocation of satisfactory reaction the monkeys became refractory to new sensitization by reagents, even after the lapse of 6 weeks and using sensitization of another cutaneous area. After incubation at 56°C for one hour, serum from allergic subjects lost its ability for passive cutaneous sensitization of monkey skin.

On passive transfer to human skin—Prausnitz-Küstner technique—reagents were demonstrated in 29 out of 53 sera from persons allergic to penicillin.

Sodium benzylpenicillin, 2,000,000 units, administered intravenously to the human recipients, elicited positive reactions in 15 sera (Tables 1 and 3). At this dosage there was no difference in the results obtained with commercial and with Sephadex-purified penicillin.

TABLE 3

Specificity of Reagents in Sera from 29 Penicillin Allergics to Benzylpenicillin, the Penicilloyl radical, and Benzylpenicilloate Respectively Demonstrated by the Prausnitz-Küstner Technique.

Number of patients	Specificity of reagents
7	Penicillin
2	Penicillin + Penicilloate
2	Penicillin + Penicilloyl
4	Penicillin + Penicilloyl + Penicilloate
8	Penicilloyl
6	Penicilloyl + Penicilloate

Penicilloyl-Polylysine "Cilligen" PPL 50 ($50 \times 10^{-5}\text{ M}$) 1.2 ml, administered slowly by the intravenous route to the

human recipients, elicited positive reactions in 20 sera (Tables 1 and 3).

Sodium benzylpenicilloate (1.7×10^{-2} M) 0.05 ml, injected intracutaneously into the human recipients into each sensitized field, elicited positive reactions in 12 sera (Tables 1 and 3).

Just like the passive transfer to monkey skin, the Prausnitz-Küstner technique often showed a positive reaction to different antigens in the same serum (Table 3).

25 human recipients were satisfactorily sensitized. The results were very dependable from recipient to recipient, and the sensitization failed in only one human recipient. This person was a non-allergic who gave normal reaction to intracutaneous injection of 0.05 ml injectable histamine chloride 0.01 per cent.

Titration of the reagin activity in the serum samples was done only on human skin and with all positive sera immediately after dilution with normal saline. To restrict the number of injections into the recipients the titration was performed for serum dilutions 1:1, 1:10, 1:100, 1:1000, and 1:10,000. The titration was carried out in duplo on different recipients independently for each of the 3 antigens. Reagin titres of 1:1 to 1:1000 were demonstrated for penicillin and the penicilloyl radical and of 1:1—1:100 for penicilloate (Table 1).

The *antigen amounts* used in the passive transfere tests to monkey skin and in Prausnitz-Küstner tests were sufficient to elicit maximal responses.

DISCUSSION

Skin-sensitizing antibodies were demonstrated by the Prausnitz-Küstner technique in 29 out of 53 sera from patients with proven penicillin allergy. Positive reactions could be provoked by benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate. Similar results have been found by others (Redmond & Levine 1967).

No sera reacted exclusively to benzylpenicilloate, but the fortuitous distribution of positive reactions to one or more of the 3 chemical compounds suggested that there was a question of 3 specific and different reactions. The results of titrating the reagin content in the sera also indicated the presence of 3 different, specific antigens in penicillin allergy. Thus, a high titre of reagins obtained by one antigen did not necessarily entail a positive reaction to one of the other antigens.

Identical results were obtained with commercial and purified penicillin in the experiments suggesting that all the reactions provoked by commercial penicillin were penicillin-specific.

A comparison of the results of passive transfer to monkey skin with those of passive transfer to human skin showed the latter to be more sensitive. Out of 29 sera shown to contain reagins by the Prausnitz-Küstner technique only 16 could elicit reaction upon passive transfer to monkey skin. With two exceptions a positive reaction could be elicited in monkeys only when the reagin titre on human skin was at least 1:10. All sera having reagin titres of 1:100 and over usually gave positive reactions upon passive transfer to the skin of satisfactorily reacting monkeys.

No serum gave a positive reaction upon passive transfer to monkey skin without simultaneous reaction upon transfer to human skin, and the two tests always showed the same specificity.

Accordingly, passive transfer to monkey skin seems less suited for the diagnostic demonstration of skin-sensitizing antibodies in serum from patients with penicillin allergy. The method is not sufficiently sensitive, and in several sera the reagins are of such low titre that they are demonstrable only upon passive transfer to human skin. Furthermore only 35 per cent of randomly selected monkeys can be satisfactorily sensitized by human reagins. However, the test may no doubt be applicable for other objects, *e.g.* for determining possible cross allergy between various penicillin derivatives or between penicillin and other antibiotics. Such studies presuppose the

use of sera with sufficiently high reagin titre, 1:100 or over when determined by the Prausnitz-Küstner technique.

By the Prausnitz-Küstner technique reactions to penicilloyl compounds were satisfactorily elicited in human recipients by intravenous injection of small quantities of penicilloyl-polylysine, 1.2 ml 5×10^{-4} M. Reactions to penicillin have previously been provoked under similar conditions, by intravenous injection of penicillin in quantities of the same magnitude 1.0 ml 8.5×10^{-4} M (*Pedersen-Bjergaard* 1967), although larger quantities of penicillin were used in the present study.

On the other hand, reactions to penicilloate, by the Prausnitz-Küstner technique, could not be elicited except by direct intracutaneous injection into the sensitized site of 0.05 ml 1.7×10^{-2} M benzylpenicilloate. This is a striking difference between the reactions to penicillin and penicilloyl compounds, which are provoked by small quantities of antigen intravenously, and the reactions to penicilloate, which require a far larger quantity of antigen injected locally into the sensitized site. Several explanations may be imagined. One possibility is that benzylpenicilloate as such is not the real antigen in reactions elicited by benzylpenicilloate solutions.

SUMMARY

Sera from 53 patients with proven penicillin allergy were studied. The diagnosis had been confirmed in 48 by provocation tests with penicillin.

On passive transfer to monkey skin reagins were demonstrated in the sera from 16 patients. Following sensitization the reactions could be provoked for each serum by one or more of the following preparations: benzylpenicillin, penicilloyl-polylysine, and benzylpenicilloate.

On passive transfer to human skin (Prausnitz-Küstner technique) reagins were demonstrated in the sera of 29 of the 53 penicillin allergies by the same antigens.

Sera capable of sensitizing monkey skin invariably gave a positive result on passive transfer to human skin, and the

reactions were always elicited by the same antigen in both methods. However, a reagin titre of or exceeding 1:10 on human skin was in most cases required to sensitize monkey skin. It was not until a reagin titre of 1:100 that reproducible reactions were obtainable on monkey skin.

Only 35 per cent of randomly selected young monkeys could be satisfactorily sensitized by reagin containing human serum. Thus, passive transfer to monkey skin is not suited for the serological diagnosis of penicillin allergy, reagins in the sera of penicillin allergics often being present in such low titre that they are demonstrable only upon passive transfer to human skin.

The Prausnitz-Küstner reaction was elicited by one or more of the 3 antigens, without a definite relationship between the reagin titre for one antigen and reaction to the others. This finding indicates that in human penicillin allergy at least 3 different specific antigens are operative, represented by penicillin, the penicilloyl radical, and penicilloate.

Prausnitz-Küstner reactions could be provoked by small quantities of benzylpenicillin and penicilloyl-polylysine intravenously. With benzylpenicilloate, on the other hand, reactions could only be elicited by direct intracutaneous injection of the substance in a high concentration. This difference may be due to a number of causes. For instance, it may be imagined that the benzylpenicilloate as such is not the true antigen in the reactions elicited by this substance in human penicillin allergy.

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