关键词 彩绒革盖菌；多糖；肽；T 淋巴细胞；下丘脑中部；环孢菌素类；神经免疫调制

目的：探讨云芝糖肽（PSP）免疫增强作用的神经机制 方法：用 Wistar 大鼠细胞外记录下丘脑内侧基底部（MBH）神经元的单位放电，并检测淋巴细胞的增殖作用 结果：给大鼠灌胃 PSP 1 g·kg^{-1}，连续 5 天，使脾脏 T 淋巴细胞增加，并引起脾脏和外周血中 T 淋巴细胞的明显增殖 损毁 MBH 能阻断这种增殖作用。PSP 使 MBH 神经元的放电频率增加，加免疫抑制剂环孢菌素 A 能去除这种作用 结论：神经系统（主要是 MBH）参与 PSP 的免疫增强作用。

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Enzyme-linked immunosorbent assay for detection of IgG against penicillins in children

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KEY WORDS enzyme-linked immunosorbent assay; Western blotting; haptens; IgG; penicillins; cross reactions; child

AIM: To establish an enzyme-linked immunosorbent assay (ELISA) for detection of IgG against penicillins and then investigate this antibody response in children. **METHODS**: Western blotting, ELISA, and hapten inhibition assay were used. **RESULTS**: Penicillins reacted with bovine serum albumin (BSA) to generate conjugate which was used as coating antigen (100 mg · L^{-1}) in ELISA. A good correlation was found between absorbance (A) and dilutions of sample with a linear coefficient of 0.9918. Thirteen subjects who were clinically suspicious of penicillin allergy were tested for specific IgG by ELISA, 4 positive for benzylpenicillin, 1 for benzylpenicillin and piperacillin, and 3 for benzylpenicillin, piperacillin and ampicillin. **CONCLUSION**: An ELISA was successfully established and penicillin reactive IgG response in children was heterogeneous. IgG antibodies recognized benzylpenicillin molecule, degraded products and new antigenic determinant from conjugate. Cross reactions occurred among benzylpenicillin, ampicillin and piperacillin.

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Penicillins are responsible for 15 % of anaphylactic deaths[1,2]. IgE against penicillins is the most frequent cause of anaphylaxis in human[3-5]. A number of *in vivo* and *in vitro* techniques have been established for the diagnosis of immediate allergic reactions to penicillins including skin test and radioallergosorbent test (RAST). For the detection of penicillin-reactive IgE antibody, RAST has been used and reported to produce excellent correlation with skin test results using penicilloyl-polylysine. In the human sera, IgG represents about 80 % of total immunoglobulins, its half-life being much longer than IgE. However, limited information is available concerning the role of specific IgG against penicillins in penicillin allergy. During the course of surveillance of adverse drug reactions, we wanted to establish an enzyme linked-immunosorbent assay (ELISA) to detect penicillin-reactive IgG in children.

MATERIALS AND METHODS

Subjects and sera Sera were collected within 1 wk after the occurrence of allergic symptoms from 13 patients, aged 3.0 ± 2.7 a and weighing 12.5 ± 7.6 kg, who were clinically suspicious of penicillin allergy in Pediatric Hospital, Shanghai Medical University. Sera from 10 non-allergic patients who had not received penicillins, aged 3.2 ± 2.6 a and weighing 13.1 ± 8.2 kg, were also collected as control.

Reagents Bovine serum albumin (BSA) was obtained

from Sigma Chemical Co (USA), horseradish peroxidase-labelled sheep anti-human IgG from Gibco BRL (USA), 96-well polystyrene microtitre plates from Coster (USA). Benzylpenicillin, ampicillin, and piperacillin were obtained from Shanghai № 3 Pharmaceutical Factory.

ELISA for detection of IgG against penicillins Penicillins and BSA (wt/wt 10:1) were dissolved in carbonate buffer 0.05 mol·L^{-1} (pH 10.5) and final concentrations were 1 g·L^{-1} and 100 mg·L^{-1}, respectively. The mixture was gently shaken at 37 ℃ for 24 h to produce penicillin-BSA conjugate[6,7], and coated on microtiter plates with 100 μL/well at 4 ℃ overnight. Plates were washed three times with phosphate-buffered saline 0.1 mol·L^{-1} containing 0.05 % (vol/vol) Tween-20. Prediluted sera (1:30) 100 UL were added in triplicate, then incubated at 37 ℃ for 2 h. Plates were washed, 100 μL of horseradish peroxidase-labelled sheep anti-human IgG (1:400) were added and incubated at 37 ℃ for 2 h. After washing, 100 μL of substrata solution containing O-phenylenediamine 400 mg·L^{-1} and 0.15 % H_2O_2 (30 % wt/vol) in citrate-phosphate buffer (0.1 mol·L^{-1}, pH 5.0) were added and incubated at 37 ℃ for 0.5 h. Enzyme-substrate reaction was stopped by addition of 50 μL of H_2SO_4 (4 mol·L^{-1}). Absorbance (A) was read at 492 nm using EIA Reading (Bio-Rad, USA). The mean A value and standard deviation of control sera was calculated as threshold ($t = \bar{x} + 3s$), above which the tested serum was considered as specific penicillin-reactive IgG positive.

Hapten inhibition assay The assay was performed[5] with some modification: 50 μL of prediluted serum (1:15), which was a mixed serum with strong penicillin reaction, was preincubated at 37 ℃ for 1 h with 50 μL of benzylpenicillin-BSA conjugate, freshly prepared benzylpenicillin solution or aged benzylpenicillin solution which had been stored at 37 ℃ for 6 h and at 4 ℃ for overnight, and then ELISA was performed.

Western blotting The assay was performed[8] with some modifications. BSA was incubated in carbonate buffer (pH 10.5) at 37 ℃ for 24 h and then analyzed using SDS-PAGE electrophoresis using Mini-Protean system (Bio-Rad, USA). Penicillin-BSA conjugate was separated by SDS-PAGE and then transferred onto nitrocellulose paper using Trans-Blot Cell (Bio-Rad, USA). Nitrocellulose paper strips containing the antigens were incubated with prediluted serum (1:50). After being washed, the test strips were incubated with peroxidase-labelled sheep anti-human IgG. Strips were then washed and incubated with DBA. After 10 min, reaction was stopped by washing strips with water.

RESULTS

Identification of penicillin-BSA conjugate Only 67-kDa BSA band appeared after SDS-PAGE electrophoresis, suggesting that BSA molecules did not form polymer or degraded products during incubation and that BSA was a suitable protein molecule for preparation of hapten-carrier complex. Penicillin reactive sera reacted with 67-kDa antigen, suggesting that benzylpenicillin and BSA formed conjugate and the benzylpenicillin of conjugate was recognized by specific IgG. Moreover, like benzylpenicillin, ampicillin and piperacillin also reacted with BSA to form conjugate and were recognized by specific IgG. Sera from non-allergic patients did not react with penicillin-BSA conjugate.

Effect of amounts of BSA and penicillins on ELISA Optimal conditions, benzylpenicillin-BSA (wt/wt 10:1) and coating concentration 100 mg·L^{-1} (according to the weight of BSA) were given for the ELISA (Tab 1).

Tab 1. $A_{492\ nm}$ values for coating conjugate in ELISA.

Benzylpenicillin-BSA/mg·L^{-1}	Benzylpenicillin: BSA (wt/wt) 1:1	5:1	10:1	20:1
10	0.14	0.11	0.19	0.09
50	0.13	0.08	0.34	0.40
100	0.12	0.19	0.66	0.39
200	0.13	0.23	0.57	0.35

Relationship between A values and dilutions A series of dilutions of penicillin reactive sera, from 1:5 to 1:400, were detected using ELISA. A good correlation was found between A values and dilutions with a linear coefficient of 0.9918. Sera (1:30) was suitable for ELISA (Fig 1).

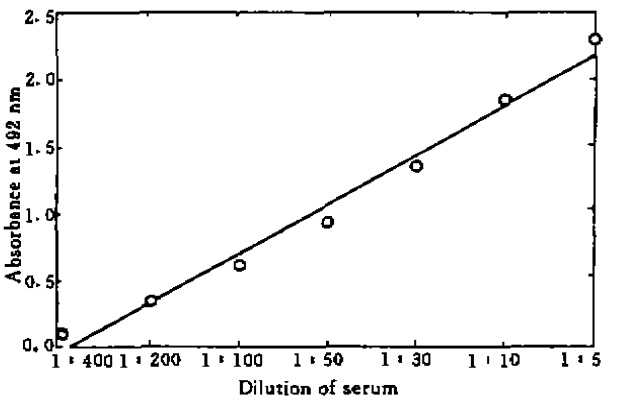

Fig 1. Dilutions of antibody against benzylpenicillin and A values.

Hapten inhibition response Detailed inhibitory dose-response profile of benzylpenicillin-BSA conjugate was almost the same as that of aged benzylpenicillin, but higher than that of freshly prepared benzylpenicillin (Fig 2).

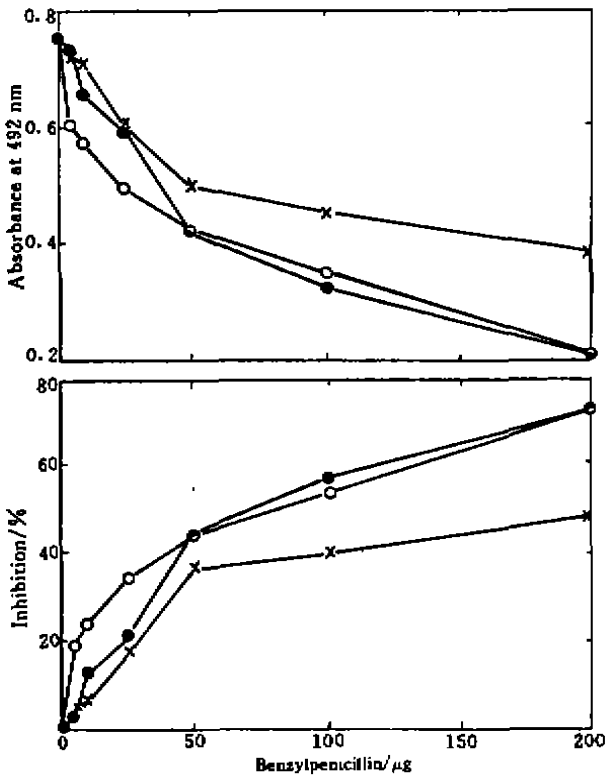

Fig 2. Hapten inhibition with benzylpenicillin-BSA (○), aged benzylpenicillin (●), and fresh benzylpenicillin (×).

Detection of IgG against penicillins By ELISA, 8 of 13 subjects were positive for IgG against benzylpenicillin, 4 of 13 for piperacillin and 3 of 13 for ampicillin. Comparison of IgG response against three kinds of antibiotics, we found 3 subjects were IgG positive for all those antibiotics, 1 subject for benzylpenicillin and piperacillin, and 4 subjects only for benzylpenicillin.

DISCUSSION

Because penicillins can react readily with proteins *in vitro* at alkaline pH, we generated successfully penicillin-BSA conjugate which was confirmed by Western blotting, and then established an ELISA for the detection of IgG against penicillins, in which BSA of conjugate was coated onto the wells of plates and penicillins of conjugate acted as antigen to react with specific combining sites of penicillin-reactive IgG in sera from allergic patients. We found that the amounts of BSA and penicillins affected the *A* values in ELISA, which supported the finding[9] that hapten density (number of drug molecules conjugated per molecule of protein) had a significant influence on antibody binding. The exact nature and origin of immunogen responsible for penicillin stimulation of immune system has not been entirely known[2,7]. For our penicillin-BSA conjugate prepared with a prolonged period of time and strong alkaline pH, we believed that the antigenic determinants of conjugate should include not only penicillin molecule itself but also penicillin polymer, degraded and transformation products. Permitting the optimal combination of high sensitivity of enzymatic reaction and high specificity of immunologic reaction, our ELISA system needed only 7 μL of serum, so this assay may be especially suitable for research in children. This immunoenzymatic technique was also suitable for detection of antibodies against ampicillin and piperacillin.

We drew out some conclusions from the inhibition experiment: 1) a smaller part of IgG antibodies were against new antigenic determinant from the binding of benzylpenicillin to BSA, because conjugate showed higher inhibitory activity than aged benzylpenicillin and freshly prepared benzylpenicillin when concentrations of benzylpenicillin were 0 – 50 μg. 2) freshly prepared free benzylpenicillin did show inhibitory activity although its activity was weaker than that of the conjugate or aged benzylpenicillin, which clearly suggested that some parts of IgG antibodies recognized the benzylpenicillin molecule and other recognized degraded products, polymer or transformation products. The second conclusion was differ from the view[1,10] that IgE antibodies detected in the sera of patients were not directed towards penicillin itself but towards the penicilloyl hapten.

By the ELISA, we found 8 of 13 children, clinically suspicious of penicillin allergy, were positive for IgG against penicillins. Recently, the side chain specific beta-lactam allergy has received a great attention[11,12]. We thereby further studied

the immunological cross reaction among benzylpenicillin, ampicillin, and piperacillin. Our results showed that IgG antibodies from 4 subjects recognized piperacillin and benzylpenicillin, and IgG from 3 subjects bound with all three penicillins, indicating that some IgG antibodies were against the molecular nucleus of penicillins and some against side chain, since all the penicillins tested in this paper varied in side chain only. Therefore, it was clear that penicillin reactive IgG antibody response in children was heterogeneous.

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酶联免疫吸附试验测定儿童体内抗青霉素类 IgG 抗体

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关键词 酶联免疫吸附测定；蛋白质印迹；半抗原；免疫球蛋白 G；青霉素类；交叉反应；儿童

A 目的：建立一个测定青霉素类反应性 IgG 的酶联免疫吸附方法(ELISA)并用该方法观察儿童血清中的特异性抗体. 方法：采用免疫印迹、ELISA、半抗原抑制试验. 结果：青霉素类和牛血清白蛋白结合成复合物，该复合物能作为 ELISA 中的包被抗原(100 mg·L^{-1}) 样品稀释倍数和吸光度间有良好的相关性，相关系数 0 9918. 运用 ELISA 在 13 例临床可疑青霉素过敏的患儿中检测出 4 例青霉素抗体，1 例青霉素和氨苄西林抗体，3 例青霉素、氨苄西林、哌拉西林抗体. 结论：成功的建立了一个 ELISA 方法，针对青霉素的 IgG 应答在儿童具有异质性. IgG 抗体不但能识别青霉素分子本身，还能识别降解产物以及来自复合物的新抗原决定族. 青霉素、氨苄西林、哌拉西林间有免疫交叉反应.