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Hypersensitivity of Bronchial Asthmatics to Cockroach in Taiwan

Comparative Study between American and German Cockroaches

Key Words

Bronchial asthma
American cockroaches
German cockroaches

Abstract

This study was performed to test the hypersensitivity of asthmatics to American and German cockroaches, which are both common in Taiwan. A total of 236 asthmatic patients received skin prick test using allergen extracts from both American and German cockroaches, and 596 sera from asthmatic patients were analyzed for their specific IgE against German cockroach extract. The results of skin test showed that 39.4 and 36.4% asthmatic patients were hypersensitive to American and German cockroaches. Fifteen among 236 patients were only allergic to American cockroaches and 8 were only allergic to German cockroaches. Using the Pharmacia CAP system, 36% of the sera were found to contain the specific IgE to German cockroach extract. Eighty-nine sera positive for German cockroach extract were then tested for their reactivity to American cockroach extract using the fluoroallergosorbent test (FAST). Sixty among 89 (68%) of their sera contained American cockroach-specific IgE. The correlation coefficient between both parameters was $r = 0.45$. Immunoblot and immunoblot inhibition studies were performed to analyze the IgE-binding components and the cross-reactivity between American and German cockroaches. The results showed that there are different IgE-binding components between American and German cockroaches. Sera containing specific IgE to both species of cockroach were absorbed with both species of cockroach extracts. The specific IgE to German cockroaches can be absorbed by American cockroach extract in all selected sera and the specific IgE to American cockroaches can only partially be absorbed by German cockroaches. The nonabsorbed allergens in American cockroaches had molecular weights of 33 and 50 kD. In conclusion, one-third of the asthmatic population tested was allergic to cockroaches. Although most cockroach-hypersensitive patients were allergic to both American and German cockroaches, more asthmatic patients were allergic to American cockroaches in Taiwan. The use of non-cross-reacting allergen in detecting American cockroach-specific IgE might be important not only for the diagnosis and treatment of cockroach hypersensitivity in asthmatics but also for the differentiation between German and American cockroach hypersensitivity.

Introduction

Cockroaches have been identified as a major cause of perennial rhinitis and asthma [1–5]. High incidences of positive skin tests to cockroaches in atopic (20–53%) and asthmatic populations (49–61%) have been reported [6, 7]. Hypersensitivity to American cockroaches has been reported in Taiwan with a prevalence rate very similar to that in the United States. Among 182 allergic patients in Taichung, 54.9% exhibited a positive immediate skin reaction to an intracutaneous challenge [8]. A recent multicenter study of the home environment of children with asthma living in seven major urban areas of the United States revealed that cockroach allergen was present in 89% of bedrooms, while mite allergen was detectable in only 49% of bedrooms. Therefore the major allergen of cockroaches, Bla g 1 was considered to be the predominant indoor allergen in the inner cities of United States [9]. In Taiwan, the warm and humid semitropical weather, the high population density, and unsatisfactory sanitary conditions may contribute to the prevalence of cockroaches in the household. According to the consulted entomologists, both American and German cockroaches are common in Taiwan [8], and it has been suggested that there are common IgE-binding components in whole-body extracts of American and German cockroaches [10–14]. The cross-reactive allergens appear to have molecular weights of 60, 52, 49, 38 and 12 kD [15]. The species-specific allergens, Bla g 2, Bla g 4 and Bla g 5, of German cockroaches and Per a 3 (Cr-PII) of American cockroaches have recently been cloned and sequenced [16–19], the non-crossreacting allergens of American cockroaches have not been investigated. Therefore, it is of interest to compare hypersensitivity to American cockroaches with hypersensitivity to German cockroaches in asthmatic patients and to investigate the specific allergens in these two cockroaches using sera from asthmatic patient. Patients with asthma were tested against commercially prepared American and German cockroach allergens. The specific IgE was determined by using the Pharmacia CAP system for German cockroaches and the FAST system for American cockroaches. The allergenic properties and cross-reactivity between American and German cockroaches were also investigated by immunoblot inhibition analysis. In this study, we have identified the non-cross reacting allergens of cockroaches and clarified the relationship between cockroach hypersensitivity and asthma.

Materials and Methods

Patients

A total of 596 asthmatics was selected from the Allergy Clinic at Cathay General Hospital in Taipei, Taiwan. The selection criteria for patients with bronchial asthma were based on the criteria of the American Thoracic Society for asthma [20]. All patients with asymptomatic asthma and/or allergic rhinitis underwent a skin prick test and specific IgE measurement. The age distribution of patients ranged from 3 to 70 years.

Skin Prick Test

The skin prick test was performed using a commercial disposable needle (Greer Derma Pik system; Greer Laboratories, Lenoir, N.C., USA). All allergen extracts and controls were obtained from Greer Laboratories. After dipping with Derma Piks each allergen extract was applied on the volar surface of the patients left forearm. The distance between each prick site was 20 mm. Allergen extracts used for prick testing were American cockroach (1:10 w/v), German cockroach (1:10 w/v), *Dermatophagoides pteronyssinus* (30,000 AU/ml), histamine phosphate (1 mg/ml) and 50% glycerin solution alone. Fifteen min later, the reactions were read and the sizes (in millimeters) of wheal and flare were recorded. Wheal sizes equal to or greater than 3×3 mm were considered positive, but only if there were no reactions from the negative controls. The interpretation was recorded according to the following criteria: (–) wheal <3 mm in diameter, (1+) wheal 3–6 mm, (2+) wheal 6–10 mm, (3+) wheal 10–15 mm and (4+) wheal >15 mm with pseudopods.

Measurement of Allergen-Specific IgE

The Pharmacia CAP system was used for all specific IgE determinations (Pharmacia, Sweden) except for American cockroach extract. Serum samples were incubated in an allergen-coated solid phase consisting of a hydrophilic carrier polymer encased in a capsule, the immuno-CAP, for 30 min. The immuno-CAP was then dispensed onto a microplate and placed in an assay processor that automatically performed all the successive steps, such as incubation, washing and addition of reagents. The solid phase provides favorable reaction conditions, so equilibrium can be reached in less than 20 min. After washing, a combination of β -galactosidase-labeled polyclonal and monoclonal anti-IgE sera was added and incubated for 150 min. After further washing, a development solution was added and incubated for an additional 10 min. After addition of the stop solution, the microplate was placed onto the Fluoro Count 96 for measurement. Results were expressed in KU/l (with calibration against the WHO standard for IgE); the concentration range was from 0.35 to 100 KU/l. The titers of specific IgE were further classified as follows (KUA/l): class 1: 0.35–0.7, class 2: 0.7–3.5, class 3: 3.5–17.5; class 4: 17.5–50.0, class 5: 50–100, and class 6: >100.

Measurement of American-Cockroach-Specific IgE

Serum IgE antibodies to American cockroach extract were determined by the FAST using an enzyme immunoassay kit (IgE FAST-plus Test, 3M; Diagnostic System Inc., Santa Clara, Calif., USA); the test was performed according to the manufacturer's instructions. Optimal concentrations of American cockroach extracts (4 μ g/well) were determined by checkerboard titration and extract was coated onto blank microtitration wells (3M; Diagnostic System Inc.) in the laboratory. 50 μ l of serum was added to the coated wells and incubated for 2 h at room temperature. Alkaline-phosphatase-labeled monoclonal

anti-human IgE conjugate was added and incubated at room temperature for 2 h. Excess enzyme-labeled anti-IgE was washed away, and fluorescence substrate (4-methylumbelliferyl phosphate in 2-amino-2-methyl propanol buffer, pH 9.5) was then added into the wells which were then incubated at room temperature for 40–60 min. After incubation, IgE concentration (IU/ml) was determined using a 3M Diagnostic System fluorometer with calibrators containing 0.35, 0.8, 4.0 and 20.0 IU/ml (1.0 IU/ml = 2.4 ng/ml) perennial ryegrass-specific IgE as standards.

Immunoblot Analysis

The immunoblot analyses of cockroach allergens recognized by human IgE antibodies were carried out according to a standard method [13]. Cockroach extracts (0.52 mg/ml), obtained from Greer Laboratories, were diluted with an equal volume of SDS-PAGE sample buffer (containing 2% SDS and 5% 2-mercaptomethanol) and boiled for 5 min, then separated on a 12.5% SDS-polyacrylamide gel and transferred electrophoretically onto polyvinylidene difluoride (PVDF) membrane (0.45 µm, Millipore, Bedford, Mass., USA). After blockade with 1% skim milk in Tris-buffered saline (TBS, 20 mmol/l of Tris, 500 mmol/l of NaCl, pH 7.5), the blots were incubated with diluted serum samples (1:5 dilution in TBS containing 0.05% Tween 20 (TBST) and 1% skim milk) for 16 h at 4°C. After washing with TBST, the blots were incubated with alkaline-phosphatase-conjugated monoclonal anti-human IgE antibodies (Pharmingen, San Diego, Calif., USA) for 1.5 h at room temperature. Following washing procedures, the blots were developed in a substrate solution of carbonate buffer (0.1 M NaHCO₃, 1.0 M MgCl₂, pH 9.8) containing 0.35 M 5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt and 0.37 M p-nitrobluetetrazolium chloride for about 30 min. After washing with water, deposition of the purple stain which demonstrates antigen-antibody binding was recorded by photography. For control, serum samples obtained from cord blood were used.

Immunoblot Inhibition

To test the cross-reactivity between American and German cockroach extracts, immunoblot inhibition was performed. An IgE-positive undiluted serum sample was mixed individually with an equal volume of American (0.52 mg/ml) or German cockroach extract (0.52 mg/ml) by end-over-end rotation at 4°C for 1 h. The PVDF blots were then incubated individually with the IgE-positive serum or a different serum-extract mixture at 4°C for 16 h. The blots were washed and incubated with alkaline-phosphatase-conjugated monoclonal anti-human IgE antibodies and developed with the enzyme substrate as described above. As a positive control, the same antigens were used in the absorbed solution and PVDF membranes.

Statistical Analysis

A linear regression study was used to test the relationship between the titers of German and American cockroach-specific IgE.

Results

A total of 596 sera from patients with bronchial asthma were tested for their content of allergen-specific IgE against 6 commercially prepared allergens (Pharmacia). The percentage of IgE-specific antibodies was highest to *D.*

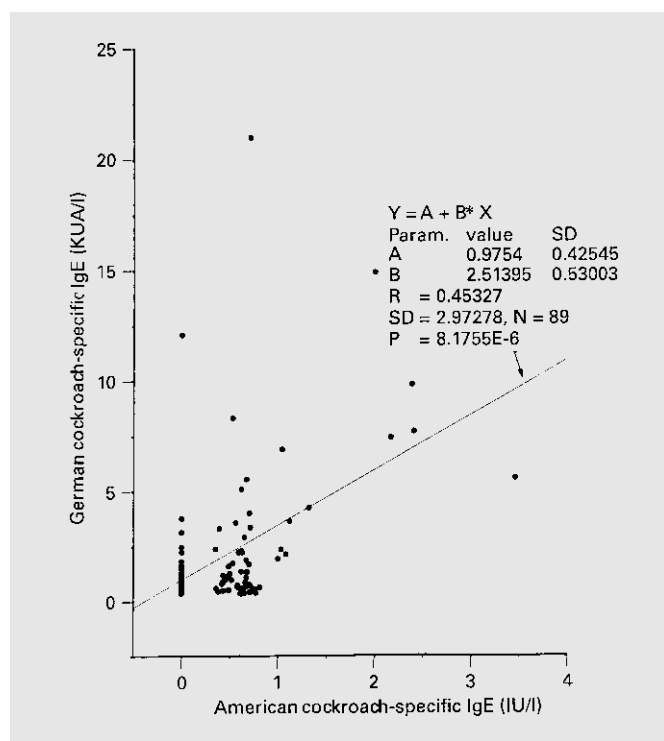


Fig. 1. Correlation between the titers of German and American-cockroach-specific IgE.

Table 1. The response rate of skin prick tests in atopic asthmatic patients (n = 236)

	Patient number	Percentage
American cockroach	93	39.4
American cockroach only	15	6.4
German cockroach	86	36.4
German cockroach only	8	3.4
Both positive	78	33.0
Both negative	135	57.2

pteronyssinus (481/596, 81%), followed by *D. farinae* (288/364, 79%), German cockroaches (187/526, 36%), cat dander (70/328, 22%), dog dander (50/362, 8%), and lowest to Bermuda grass (19/353, 5%).

There were 39.4% (93/236) and 36.4% (86/236) skin prick test positive reactions to American and German cockroaches. The results of skin prick tests were as follows: 34% were 1+, 46% were 2+, 14% were 3+ and 4% were 4+. Although most patients were allergic to both species of cockroach, 6.4% (15/236) were only allergic to American cockroaches and 3.4% (8/236) only to German cockroaches (table 1). When both skin prick test and Pharmacia CAP IgE

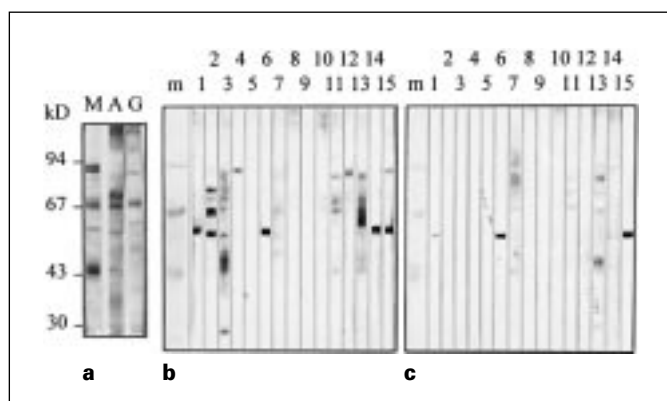


Fig. 2. IgE binding component of cockroach extract analyzed by SDS-PAGE immunoblot. **(a)** shows the Coomassie blue staining of protein markers and components of American cockroach (A) and German cockroach extracts (G). The same sera (1–5) were used to detect IgE-binding components of American cockroach extract **(b)** and German cockroach extract **(c)** on the PVDF blot. Lane M shows the protein markers on the PVDF blot.

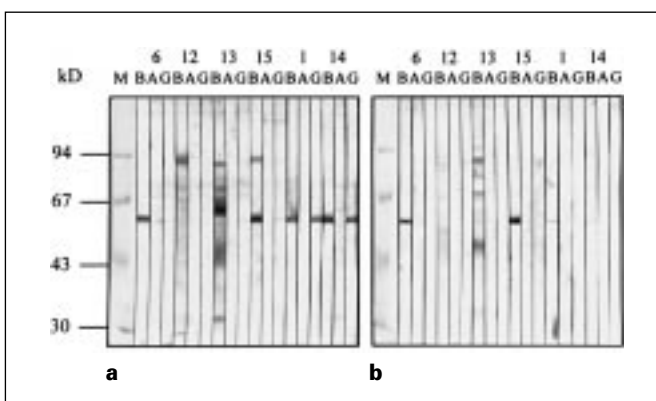


Fig. 3. Cross-reaction of American and German cockroach extract. Patient sera with positive reactions to both species of cockroach (6, 12, 13, 15, 1, 14) were selected from figure 2 and mixed with American or German cockroach extract or buffer and then analyzed immunoblotting with their IgE binding to the PVDF blot of American cockroach **(a)** and German cockroache extract **(b)**. Lane B: absorption by buffer lane A: absorption of American cockroaches; lane G: absorption of German cockroaches; lane M: markers on the PVDF blot.

measurement were used to analyze hypersensitivity to German cockroach in 65 asthmatics patients, the results showed that 20% (13/65) of specific IgE-positive asthmatic individuals were not detected by skin prick test and 18% (6/33) skin-prick-test-positive asthmatics were not detected by IgE measurement.

To test the relationship between the IgE titers for German and American cockroach extracts, 88 sera from asthmatic patients which contained German cockroach-specific IgE were selected for American cockroach-specific IgE determination. American cockroach-specific IgE could be detected in 68.2% of the sera. The correlation coefficient between the two parameters was $r = 0.45$ (fig. 1).

Immunoblotting to identify IgE-binding components in extracts of American and German cockroaches were performed. Fifteen serum samples, from asthmatics with positive skin tests to both species of cockroach were tested for the IgE binding components of cockroaches. The results showed that 13 sera contained IgE-binding activity to American cockroach; however, only 8 among 15 contained IgE binding activity to German cockroaches. There were different IgE binding components in the two species of cockroaches based on the molecular weights (fig. 2). Six sera (fig. 3) with positive reactions to both species of cockroach (6, 12, 13, 15, 1, 14) were selected for immunoblot inhibition studies. Figure 3a and 3b present the sera that reacted with the PVDF blot of American and German cock-

roaches, respectively, after mixing with cockroach extracts and buffer (strip B for buffer, strip A for American cockroaches and strip G for German cockroaches). The results show that sera with specific IgE to German cockroach extract can be absorbed completely by American cockroach extract. However, sera with specific IgE to American cockroach extract can only partially be absorbed by German cockroach extract. Two sera which only reacted with American cockroach extract were also selected for the immunoblot inhibition study. The results show that the IgE-binding activity could not be absorbed by German cockroach extract with molecular weights of 33 and 50 kD (fig. 4). There were no sera which only reacted with German cockroach extract in this study. Two other sera from patients hypersensitive to American cockroaches were also selected to investigate cross-inhibition between cockroaches and *D. pteronyssinus*. The results demonstrated that cockroach-specific IgE antibodies could not be absorbed by *D. pteronyssinus* extract (fig. 5).

Discussion

We screened a population of asthmatic patients with skin prick tests and specific IgE to determine cockroach hypersensitivity in Taiwan. One hundred eighty-seven among 526 (36%) of the asthmatic patients had German cock-

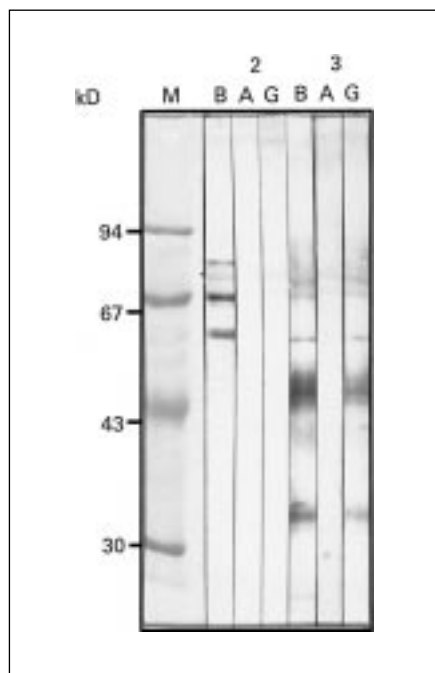


Fig. 4. Immunoblot inhibition of German cockroaches on the binding activity of American cockroach-specific IgE. Patient sera which only reacted to American cockroach extract (2, 3) were selected from figure 2 and mixed with American, or German cockroach extract or buffer and then analyzed by immunoblotting with their IgE binding to the PVDF blot of American cockroach extract. Lane M: markers on the PVDF blot. Lanes B, A, G: results after the absorption of buffer, and American and German cockroach extracts.

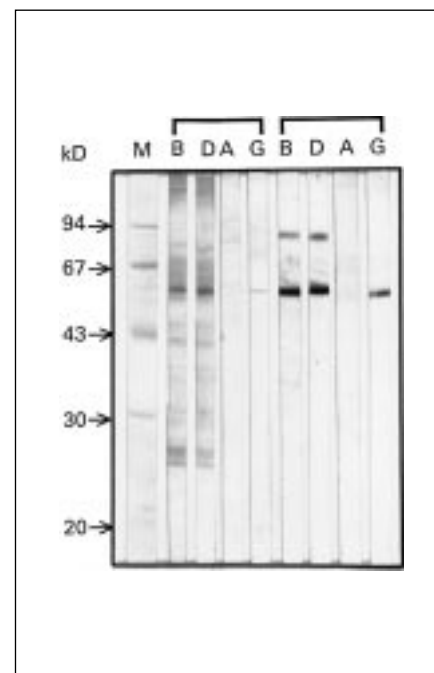


Fig. 5. Immunoblot inhibition of German cockroach and *D. pteronyssinus* extracts on the binding activity of American cockroach-specific IgE. Two cockroach-hypersensitive patients' sera were selected and mixed with buffer (B), *D. pteronyssinus* a (D), and American (A) and German (G) cockroach extract and then analyzed by immunoblotting with IgE binding to the PVDF blot of American cockroaches. Lane M: markers of the PVDF blot.

roach-specific IgE. Ninety-three and 86 among 236 skin-prick-test-positive patients reacted to American and German cockroach allergen extract. Our previous studies have demonstrated that 39.2% of asthmatic contained American cockroach-specific IgE in Taiwan [8]. These results indicated a high prevalence of cockroach hypersensitivity in Taiwan. Hypersensitivity to both American and German cockroach may play an important role in the pathogenesis of asthma in Taiwan.

Skin prick testing is a direct and safe procedure for clinical evaluation but it may also be a dreadful experience for small children. The Pharmacia CAP specific IgE measuring system was reported to be more sensitive than the PhRAST and MAST systems [19]. We used these two tests simultaneously to evaluate hypersensitivity to German cockroach extract. The results showed that the two tests were correlated. However, 20% of specific-IgE-positive asthmatics could not be detected by the skin prick test and 18% skin-prick-test-positive asthmatics could not be detected by spe-

cific IgE measurement. This discrepancy might be either due to the different cockroach antigen preparation used for skin prick tests and the Pharmacia CAP system or due to different sensitivity and specificity of these two testing methods [12]. The immunoblot results showed that not all sera had IgE-recognizing components in American and German cockroach extracts. Some showed negative results probably due to low titers, since all of the patients whose sera were used showed positive skin tests. Most of the bands in the immunoblot experiments were at 60 kD, which would agree with the 60 and 52 kD molecular weights reported for cross-reactive allergens [15].

Our previous reports and others suggested that crude extracts from German and American cockroaches contained common IgE-binding components [10–15]. In this study, most patients showing positive skin tests reacted to both American and German cockroach extracts and many sera from these patients also contained specific IgE to both species of cockroaches. This might be due to the cross-re-

acting antigenic components in both species of cockroach. According to the consulted entomologist, both species of cockroach are commonly found in Taiwan. The simultaneous reaction to both species of cockroach might also be due to the fact that our patients had been sensitized to both species of cockroaches. This cannot be clarified until the non-crossreacting allergen has been determined.

The fact that 15 among 236 asthmatic patients skin prick tested only reacted to American cockroach extract and 8 only reacted to German cockroach extract indicates that there were species-specific allergens in these two species of cockroach. Sera from these patients were used for the immunoblot inhibition study. The results showed that the titers of the specific IgE to German cockroaches were very low and could be absorbed completely by American cockroach extract; the titers of the specific IgE to American cockroaches were high and could be only partially absorbed by German cockroach extracts. These results suggest that American cockroaches play a more important role than German cockroaches in causing cockroach hypersensitivity in Taiwan. The nonabsorbed antigens in the American cockroach extracts should be further characterized and included in the test to differentiate the specific cockroach hypersensitivity. Since the Pharmacia CAP IgE measurement can only detect German cockroach hypersensitivity, it might underestimate the cockroach hypersensitivity in our asthmatic patients, since most of the patients were allergic to both species of cockroach and only few of them were allergic to either one of the two species of cockroach. This suggests that the few patients allergic only to American cockroach may have IgE antibodies only to Per a 3 or another species-

specific allergen, and not to the major cross-reactive allergen Per a 1. There were also patients allergic only to the German cockroach, suggesting that those patients have IgE antibodies against the species-specific allergens Bla g 2, 4, 5 or 6 from German cockroach. These four allergens seem to play a minor role in German cockroach allergy in the studied patients. Despite being species-specific, these allergens seem to be absorbed also by the American cockroach extracts. This fact may be mainly due to the low titer of the IgE antibodies against them, and not to any cross-reactive phenomenon. The IgE binding activity in sera 2 (fig. 4) was absorbed by German cockroach extract which might be also due to the low titer of IgE antibodies in the sera. Whether the nonabsorbed 33-kD and 50-kD allergens correspond to Per a 3 needs further clarification. The immunoblot inhibition of *D. pteronyssinus* on the binding activity of American cockroach-specific IgE demonstrates that there were cockroach-specific antigens which had no cross-reactivity between mite and cockroaches.

In conclusion, although most cockroach-hypersensitive patients were allergic to both American and German cockroaches, more asthmatic patients were allergic to American cockroaches in Taiwan. The non-crossreacting allergen in American cockroach might be important for the diagnosis and treatment of cockroach hypersensitivity in asthma.

Acknowledgements

This study was supported by a grant NSC 87-2314-B-281-001 from the National Science Council and by a Grant DOH 86-HR-508 from the Department of Health, Taiwan.

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