

EXAMINATION OF SERUM IGE SPECIFIC TO PIG PROTEIN IN PIG FARMERS BY HISTAMINE RELEASE TEST

Martin Iversen¹, Svend Norn²

¹Department of Respiratory Diseases, University of Aarhus, University Hospital of Aarhus, Aarhus, Denmark

²Department of Pharmacology, University of Copenhagen, The Panum Institute, Copenhagen, Denmark

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Abstract: Pig farmers are susceptible to a number of occupational hazards which may lead to respiratory symptoms. Therefore, inhalation allergy to pig was examined in pig farmers, including 40 farmers with work-related respiratory symptoms and 40 farmers without these symptoms. The presence in serum of IgE specific to pig protein was examined by the histamine release test, based on passive sensitization of basophil leukocytes with the farmers' serum. This test showed pig-specific IgE in a highly selected group of pig farmers in a previous study. In the present study of nonselected farmers, no swine-specific IgE was found in their serum. The results are thus in accordance with previous studies of nonselected populations of pig farmers tested by RAST and skin prick test. It can therefore be concluded that IgE-sensitization to pig protein is not a common phenomenon in pig farmers.

Address for correspondence: Svend Norn, PhD, Department of Pharmacology, University of Copenhagen, the Panum Institute, Blegdamsvej 3, 2200 Copenhagen N, Denmark. E-mail: fisn@farmakol.ku.dk

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INTRODUCTION

Working within pig farming is a recognized risk factor for the decline in lung function [6, 24, 26] and for the development of asthma-like, work-related respiratory symptoms [4, 8]. The dust level in pig confinement units is high, and analyses of dust samples suggest a high content of animal proteins [3]. Therefore, there has been considerable interest in a possible sensitization of farmers to pig proteins as a cause of the respiratory symptoms. Generally, the prevalence of positive skin prick tests and specific IgE have been very low, typically a few percent [7, 9, 10, 13, 18]. The purpose of this study was to examine if serum from pig farmers showed evidence of sensitization to pig proteins demonstrated by the histamine release test after passive sensitization of leukocytes with the patients' serum. This method may be able to demonstrate sensitization to pig protein as found in a previous study [16].

Therefore, the testing was performed in accordance with the earlier investigation [16].

MATERIALS AND METHODS

Study population. Random samples of sera were obtained from a previous cross-sectional study of 124 pig farmers [10, 11]. The mean age was 43 years, and all the farmers had worked within farming since their youth. Work-related respiratory symptoms included shortness of breath, wheezing and dry cough during work. Random serum samples stored at -80°C were selected for analysis, i.e. 40 of 48 persons with these symptoms and 40 of 76 persons without these symptoms. The study was in accordance with the second declaration of Helsinki and was approved by the local ethics committee.

Antigens. Pig protein was used, i.e. pig epithelium extract (Soluprick, 1 mg protein/ml, ALK, Denmark) and

pig urine obtained directly from the bladder and then dialyzed. House dust mite, grass pollen (Timothy, *Phleum pratense*) and cat dander, all Soluprick-SQ, 10 HEP, from ALK Denmark, were included. Anti-IgE sepharose was prepared by coupling of anti-human IgE (immunoglobulin fraction of rabbit antiserum A0094, DAKO A/S, Denmark) to CNBr-activated Sepharose 4B according to Pharmacia Biotech (Sweden).

Leukocytes. Umbilical cord blood was drawn in a heparin glass shortly after delivery, and 5 ml blood was mixed with 30 ml 0.9% NaCl including 0.3 mg/ml human serum albumin. Leukocytes including basophils were obtained by Percoll gradient centrifugation. 12.5 ml Percoll (specific gravity 1.080) was placed below the mixture of blood/0.9% NaCl. After centrifugation (296 g, 40 min), the cotton wool-like interphase of leukocytes was withdrawn, washed twice and resuspended in Tris-AMC containing 25 mM Tris at pH 7.6, 0.12 M NaCl, 5 mM KCl, 0.6 mM CaCl₂, 1.1 mM MgCl₂, 0.3 mg human serum albumin/ml and 3 µl/ml heparin (Leo, Denmark 5000 IE/ml).

Passive sensitization. Serum IgE specific to pig epithelium and pig urine was verified by passive sensitization of cord-blood leukocytes with the farmers' serum. The cells were incubated with the serum for 1 hr at 37°C and thereafter resuspended in Tris-AMC [15]. To compare with nonsensitized cells, sham sensitization of the cells were performed with patient serum replaced by medium (Tris-AMC), individual control sera from healthy (nonatopic) nonfarmers or the patients' serum deprived of IgE. The IgE was removed by the following procedure. To 200 µl patient serum was added 12 µl anti-IgE sepharose and the mixture was kept rotating for 1 hr at room temperature. After centrifugation (2,600 g, 10 min) serum was separated from the pellet of IgE- anti IgE sepharose. By this procedure more than 95% of IgE, but no IgG, was removed from serum as determined by the IgE assay, Abbott (USA) and immunonephelometry (Behringwerke AG, Germany), respectively. Additionally, the assay was controlled by passive sensitization of the cells with sera from 3 patients allergic to house dust mite, 4 to grass pollen and 3 to cat dander, all showing a Magic Lite class 3–4 response.

Histamine release. Passive sensitization was measured by histamine release. To 50 µl samples of the sensitized leukocytes were added 5 µl of pig epithelium extract, pig urine or antigen extract (house dust mite, grass pollen or cat dander) in different concentrations. Pig epithelium extract was used in dilutions of 1:33, 1:100, 1:330, 1:1,000 and 1:3,300, and pig urine in the range from 1:10 to 1:10⁶. Allergen extracts of house dust mite, grass pollen and cat dander were used in the range from 1:100 to 1:1,000. All samples, in duplicate, were incubated for 40 min at 37°C, and the release of histamine from basophils was determined as described [15] and expressed as a percentage of the total histamine content of the sample. Positive histamine release test was defined by a histamine release exceeding the spontaneous release with 10% [15, 16].

RESULTS

The presence of serum IgE specific to pig protein was examined in 40 pig farmers with work-related respiratory symptoms and compared with 40 pig farmers without these symptoms. None of the 80 farmers showed positive histamine release test to pig epithelium extract or pig urine. Thus, a net histamine release < 10% was found in all farmers with or without symptoms, i.e. no difference was observed between the two groups of farmers ($P > 0.9$ by Wilcoxon-Mann-Whitney test). Figure 1 shows representative dose responses to pig epithelium extract in two farmers, one with respiratory symptoms (Fig. 1A) and the other without symptoms (Fig. 1B). The marginal release of histamine was identical with that obtained by sham sensitization, i.e. when patient serum was replaced by medium or by control serum from healthy, nonatopic nonfarmers, and the release did not differ from the

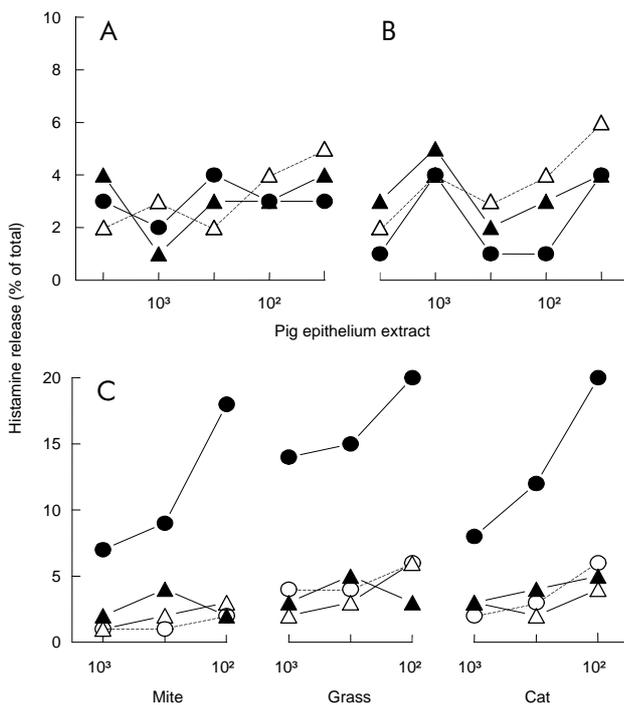


Figure 1. No swine-specific IgE was found in serum from pig farmers. IgE was measured by histamine release in response to pig epithelium extract after passive sensitization of basophils with serum from the farmers. Two examples are shown, a farmer with (A) and a farmer without (B) respiratory symptoms tested with allergen extract of pig epithelium. For comparison, three patients allergic to house dust mite, grass pollen or cat dander were tested with the respective allergen extracts (C). Passive sensitization caused by intact serum (●), serum deprived of IgE (○), medium (△) and control serum from nonatopic nonfarmers (▲) is shown. Dilution factors of allergen extracts and representative examples of various dose responses are given.

spontaneous release (not shown). Identical results were obtained by pig urine showing no significant histamine release (results not shown).

The assay was controlled in parallel experiments. Patients allergic to house dust mite, grass pollen or cat dander were tested by the respective allergen extracts. All sera from these patients showed a positive histamine release test and a representative curve of each is shown in Figure 1C, indicating the presence in serum of specific IgE. When patient serum was replaced by medium or control serum only marginal histamine release, corresponding to spontaneous release, was obtained. That was also the case by removal of IgE in serum from the patients, changing the response from positive to negative.

DISCUSSION

The question whether inhalation allergy to pigs exists has been examined in some studies based on RAST tests and skin prick tests. These studies show that IgE-mediated inhalation allergy to pigs is rarely observed, since the prevalence of positive responses amounted to only a few percent [7, 9, 10, 13, 18]. However, one study with exclusively skin prick test contrasted with these findings [1], and there has been one case report of occupational asthma caused by allergy to pigs' urine [5].

The histamine release test has been used as a sensitive test in connection with type I allergy caused by aeroallergens [19, 20]. This test has shown specific IgE to pig protein in a highly selected group of farmers, employed for at least 10 years in swine confinement buildings, and selected by the presence of both self-reported asthma and hay fever [16]. Thus, the histamine release response to pig epithelium extract and pig urine was similar to that obtained by aeroallergens in type I allergic patients, i.e. comparable with that shown in the present Figure 1C, representing patients allergic to either mite, grass pollen or cat dander. Furthermore, specific IgE was found bound to the cell surface of their basophil leukocytes. Whether a sensitization to pig protein is a common phenomenon among swine farmers should therefore be reevaluated by the histamine release test. This was the aim in the present study, which included a substantial number of persons with and without work-related respiratory symptoms during work in pig confinement buildings, a patient material well-fitted for examination of specific IgE by the histamine release test. In the present study, we used exactly the same set up and criteria as in the previous study [16], but no IgE-sensitization was found in any person since none of the 80 farmers showed a positive histamine release response to pig epithelium extract or pig urine. The results are thus in accordance with the previous studies with RAST demonstrating no IgE antibodies directed against pig protein. Therefore, it should be concluded that sensitization to pig protein may occur in some individuals, but it is not a common phenomenon, and does not explain the prevalent respiratory

symptoms occurring in pig farmers during their work in pig confinement barn environment.

However, the respiratory symptoms may well be caused by dust in the swine barns, since the dust, as well as the content in dust of lipopolysaccharides (LPS), bacteria, and moulds [3, 17, 25] are able to trigger release of inflammatory mediators and cytokines, demonstrated *in vitro* [12, 14, 21, 22, 23, 28]. Furthermore, exposure of healthy controls to swine dust in the swine confinement buildings caused release of cytokines in the airways, which was measured by increased levels of IL-1 β , IL-6, IL-8 and TNF α in the alveolar lavage fluid and nasal washes [2, 27]. Additionally, the combined effect of these noxious agents causes enhanced mediator release [21, 22, 28] and the synergy may aggravate the respiratory symptoms during work in the barns.

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