

Original articles

Potential of histamine release against inhalant allergens (*Dermatophagoides pteronyssinus*) with bacterial antigens in bronchial asthma

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SUMMARY

*In the etiopathogenesis of bronchial asthma, the important role of bacterial infection is more evident every day, favoring inflammation and obstruction, that is, triggering an asthmatic response. We gathered 36 patients diagnosed of bronchial asthma with sensitization to *Dermatophagoides pteronyssinus* and 32 healthy subjects. Histamine release tests against *Staphylococcus aureus* extract alone or together with *D. pteronyssinus* were performed, and the results were contrasted with or without the presence of *S. aureus* in the nasal secretion culture. We found histamine release against *S. aureus* higher than 10% only at the highest concentration (200 µg/ml) and significantly higher in those patients with positive nasal secretion culture. Regarding histamine release against *D. pteronyssinus* in the presence of *S. aureus*, we found a release by coincubation significantly higher than the one obtained from the addition of release against *S. aureus* and release against *D. pteronyssinus*, both in patients with negative and with positive nasal culture, at concentrations of 20 and 2 µg/ml. In conclusion, we observed a potentiation of histamine release against *D. pteronyssinus* with *S. aureus* extract in patients with bronchial asthma. These findings support the important role of the bacterial infection in the etiopathogenesis of bronchial asthma and the importance of a treatment against this infection.*

Key words: Histamine release - Bacterial antigens - Potentiation of bacterial antigens - Bronchial asthma - *S. aureus*

INTRODUCTION

One of the most controversial aspects in the etiopathogenesis of bronchial asthma is the importance of the infection factor in the asthmatic response. In several studies (1-4) we have already insisted on this aspect,

and Norn *et al.* (5-8) have confirmed its importance for the mediator release. In addition, we must consider the role played by bacterial infection in the rhinosinobronchial system, favoring inflammation and obstruction, and where the immunological response will play an essential role. Through dynamics studies in recent years (9, 10) of Th1 and Th2 lymphocyte response to antigenic stimuli, the bacterial antigens compromise the Th1 route in bacterial infections, giving way to the response, and in some cases the Th2 route, with the formation of bacterial antigen-specific IgE already proven by several authors (5, 11).

The importance of frequent bacterial infection in triggering asthmatic reaction has always been confirmed in the sensitization to the mite *Dermatophagoides pteronyssinus* as well as to other inhalant antigens. This evidence was confirmed by Norn in some studies (8) in which histamine release against *Dermatophagoides* or birch pollen from basophils of asthmatic patients was proven to increase dramatically when the antigen responsible was used *in vivo* together with the bacterial antigen. This interesting discovery prompted us to study and confirm this potentiation. In recent years, in asthmatic patients and most especially in children with sensitization to *D. pteronyssinus*, we have been able to prove that immunotherapy with bacterial antigens alone, avoiding simultaneous immunotherapy with *Dermatophagoides*, resulted in a favorable clinical evolution, with the patients feeling relatively asymptomatic from the first year and the symptomatology limited to relapses. By the second year, the patient is generally asymptomatic, with few cold relapses.

In view of the foregoing, we decided to perform a study similar to the one started by Norn *et al.* We selected a group of asthmatic patients with sensitization to *Dermatophagoides* who suffered from cold processes

with positive response to antibiotics. We also thought it would be of interest to perform a nasal secretion or sputum culture in order to determine the causal germ(s) and its or their influence in potentiation.

MATERIALS AND METHODS

Thirty-six patients were selected, aged 2 to 58, and diagnosed of bronchial asthma with proven sensitization to *D. pteronyssinus*, as determined by clinical history, skin test, specific IgE determination and histamine release test against *D. pteronyssinus*. Thirty-two healthy subjects with no sensitization were used as controls.

Skin tests

Intracutaneous skin tests were performed by means of intradermal injection of 50 μ l of *D. pteronyssinus* extract (2240 BU/mo, Ifidesa Aristegui, Bilbao, Spain), measuring the papule diameter 20 min after the injections.

IgE determination

Total and specific IgE determinations against *D. pteronyssinus* were performed by means of fluoroenzyme immunoassay (FEIA, Pharmacia CAP system, Upsala, Sweden), following the manufacturer's instructions. Values greater than 0.7 kU/l were considered positive.

Histamine release test

Histamine release test was performed by means of the fluorometric method of Shore (12), automatized by Siraganian (13, 14) and modified by us (15, 16). Briefly, 400 μ l of blood were incubated with 10 μ l of *S. aureus* extract (final concentrations: 200, 20 and 2 μ g/ml). Incubations at the same concentrations were also performed with bacterial extract and 10 μ l of *D. pteronyssinus* extract (final concentration: 2822.5 BU/ml, Ifidesa Aristegui, Bilbao, Spain) at 37 °C for 15 min. Histamine release was stopped by the addition of 1200 μ l of 10 mM EDTA in 0.9% NaCl, then the tubes were centrifuged at 750 g for 10 min at 4 °C. Supernatants were stored to analyze their histamine content the next day in a Bran-Lübbe AutoAnalyzer II (Bran-Lübbe). Basal release was obtained by incubation of cells alone without any additive, and total release by rupture of the cells with 1200 μ l of 10% HClO₄. As a positive control for histamine release, anti-IgE polyclonal serum (Caltag Laboratories, San Francisco, California, U.S.A.) was used at a final concentration of 4.2 μ g/ml.

Nasal secretion culture and antibiogram

The samples for the nasal secretion culture were collected from the patients by means of a single nasal cotton swab, with samples from both nostrils. Subsequently, they were deposited in the routine culture media: blood agar, chocolate agar, Mannitol salt agar and MacConkey agar. The strain identification was performed after 48 hours, as well as the reading of the antibiogram. We studied those antibiotics considered most useful against the usual β -lactamase-producing bacterial species. The resistance to methicillin in *S. aureus* was studied by means of a disk of oxacillin (1 μ g) in an A. Müller-Hinton agar plate with 2% NaCl. The production of β -lactamase was detected through the chromogenic cephalosporin (Nitrocephin) method. In order to study the minimum inhibiting concentration (MIC) of penicillin and ceftriaxone against *S. pneumoniae*, the ϵ -test was used, as well as a disk of oxacillin (1 μ g) in an A. Müller-Hinton agar plate with 5% lamb blood.

S. aureus extract

The strain of ATCC 25923 *Staphylococcus aureus*, cultivated in shaking trypticase soy broth culture at 35 °C for 18 hours, was used. The cells were centrifuged and washed twice in 0.85% NaCl. The sediment was ruptured by means of a French press. Subsequently, it was precipitated with two volumes of cold acetone and was left in the freezer for one night (–20 °C). It was centrifuged again, washed twice with cold acetone, and vacuum-dried. Subsequently, it was resuspended in NaCl 2.5%, in a w/v proportion of 5% and stored in continuous shaking at 4 °C for 72 hours. Next, it was intensely dialyzed against filtered water. Finally, it was lyophilized and the protein concentration was determined. The lyophilization was weighed and dissolved into 0.9% NaCl to prepare working solutions for the histamine release test.

Statistical analyses

One-way ANOVA, followed by Student-Newman-Keuls tests were performed. For the paired samples, Student's *t* test was used. Correlation was carried out using Pearson's correlation coefficient. The figures used are mean $\pm$ SEM.

RESULTS

We consider it appropriate to base the exposition of the follow-up of these patients in terms of the chosen parameters and our objective as explained in the introduction on three main points, namely: a) histamine release against *S. aureus*; b) histamine release against *D. pteronyssinus* in the presence of *S. aureus*; and c) histamine release against anti-IgE.

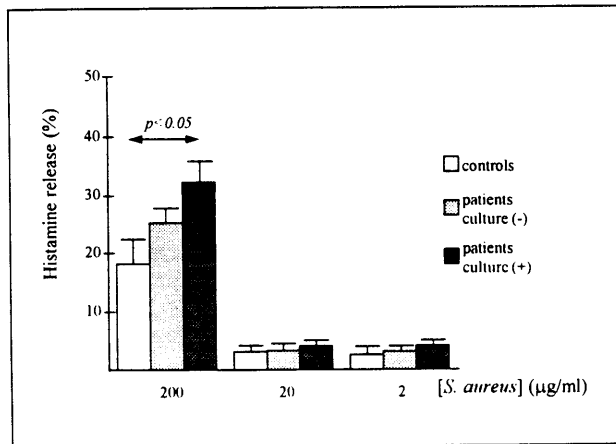


Fig. 1. Specific histamine release against *S. aureus* according to nasal culture.

Histamine release against *S. aureus*

Histamine release against the different concentrations of antigenic extract of *S. aureus* was observed, although the release was higher than 10% only at the highest concentration tested (200 µg/ml). The release at this concentration was $18.9 \pm 2.2\%$ for the healthy controls, $25.2 \pm 2.7\%$ in patients with negative nasal culture to *S. aureus* and $31.4 \pm 3.8\%$ in patients with positive nasal culture. Although releases are greater in both groups of patients, they are significantly higher ($p < 0.05$) only in patients with positive nasal culture (Fig. 1). In any case, this release of about 20% in healthy controls indicates that release at this concentration is somehow unspecific (due to nonimmunological mechanisms).

Histamine release against *D. pteronyssinus* in the presence of *S. aureus*

Histamine release against *D. pteronyssinus* at the concentration tested (2822.5 UBE/ml) was $1.6 \pm 0.5\%$ in controls, $52.7 \pm 6.8\%$ in patients with negative nasal culture and $59.8 \pm 5.6\%$ in patients with positive nasal culture.

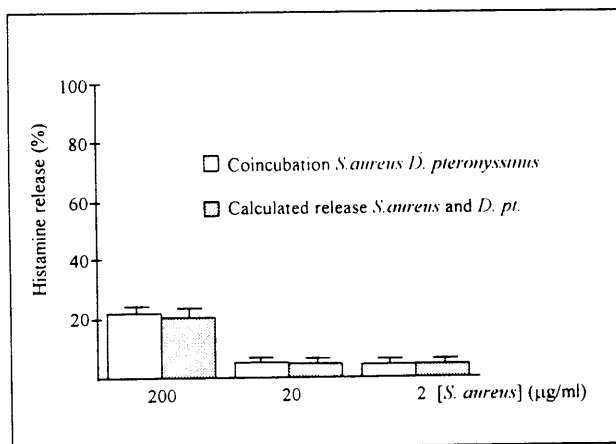


Fig. 2. Specific histamine release against *S. aureus* and *D. pteronyssinus* in controls.

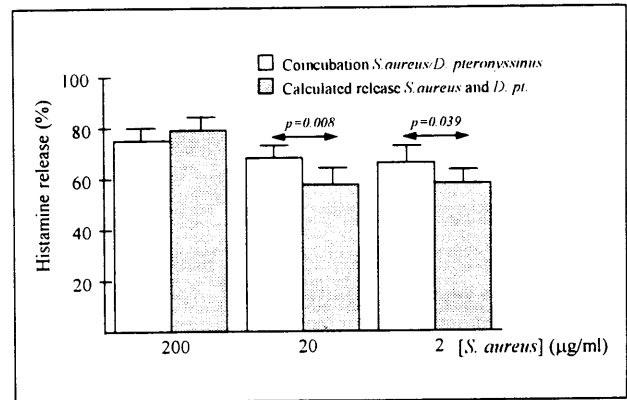


Fig. 3. Specific histamine release against *S. aureus* and *D. pteronyssinus* in patients with negative nasal culture.

There were significant differences ($p < 0.05$) between the controls and the group of patients, whereas no significant differences were found between the group of patients with negative nasal culture and the group of patients with positive nasal culture.

When the cells were incubated in the presence of *D. pteronyssinus* (2822.5 UBE/ml) and *S. aureus* at three different concentrations (200, 20 and 2 µg/ml), we obtained the results shown in Figure 2 (controls), Figure 3 (patients with negative nasal culture) and Figure 4 (patients with positive nasal culture). In order to study the possible synergistic effect, histamine release was compared with the calculated sum of histamine release by *D. pteronyssinus* and *S. aureus* in each individual.

As shown in Figure 2, there are no noticeable differences between calculated release and release caused by coincubation of both antigenic extracts. Nevertheless, as can be seen in Figures 3 and 4, both in patients with negative nasal culture and in patients with positive nasal culture, at the two lowest concentrations (20 and 2 µg/ml) of the bacterial extract, there is a potentiation in histamine release, i.e., the release obtained by coincubation of both antigens is significantly higher than the calculated sum. These differences reached significances of $p = 0.008$ and $p = 0.039$ at 20 and 2 µg/ml of *S. aureus*, respectively, for patients with negative culture, and of

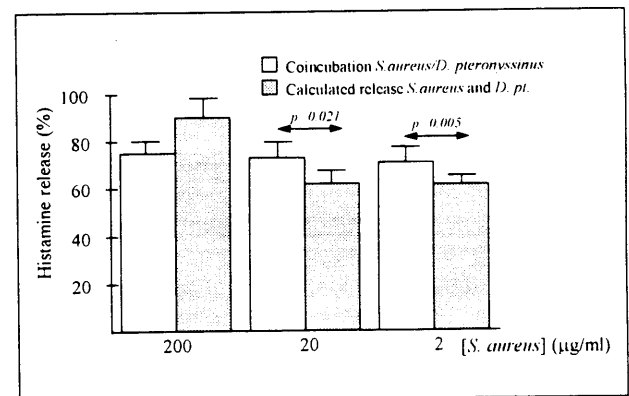


Fig. 4. Specific histamine release against *S. aureus* and *D. pteronyssinus* in patients with positive nasal culture.

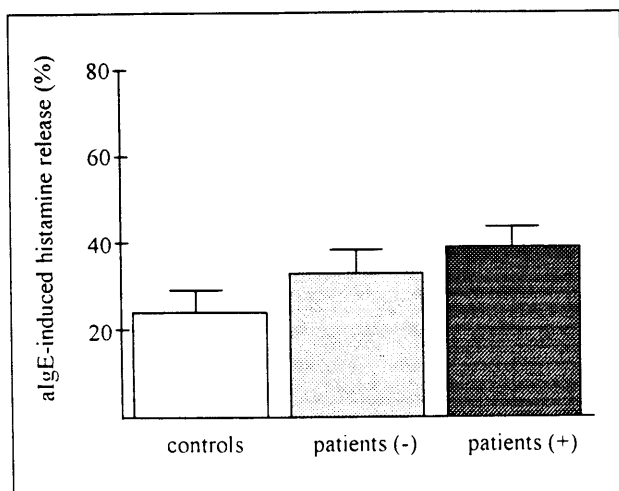


Fig. 5. algal-induced histamine release in controls, patients with negative nasal culture and patients with positive nasal culture.

$p = 0.021$ and $p = 0.005$ for patients with positive culture. It can be observed that, unlike the controls, the calculated release is higher at the highest concentration, although as we mentioned before, unspecific release mechanisms would be involved here. Figure 5 summarizes the potentiating effect of *S. aureus* given in Figures 2-4.

Histamine release against anti-IgE

Histamine release against anti-IgE antibodies was $23 \pm 5.9\%$ in controls, $33.5 \pm 5.4\%$ in patients with negative culture and $38.3 \pm 4.3\%$ in patients with positive culture (Fig. 6). Although there were no significant differences between these releases, we observed a tendency to a higher release in the patients, and within this group in

patients with positive nasal culture against *S. aureus*. These results were similar to the ones obtained in histamine release against *S. aureus* at different concentrations. A positive significant correlation was found between histamine release induced by anti-IgE antibodies and *S. aureus* used at the highest concentration ($r = 0.35$, $p = 0.012$).

DISCUSSION

In view of the results obtained and following the criteria adopted for presenting the data studied, it can be observed that our results are similar to the experience described by Norn (8). That is, the bacterial antigens play a very important role, on one hand acting as antigens with the subsequent humoral and cellular immunological response, and creating hyperreactivity of the respiratory mucosa, and on the other hand, acting as potentiators or coadjuvants of the response against inhalant allergens. This is what we observe in everyday clinical practice in asthmatic patients with concomitant infection of the rhinosinubronchial system.

The results of histamine release against the antigenic extract of *S. aureus* (Fig. 1) at the highest concentration may indirectly confirm the coexistence of two release mechanisms which participate in histamine release against bacterial antigens: a specific release, due to the antigen-specific IgE interaction, and an unspecific release, due to nonimmunological mechanisms (7, 17), which is confirmed by a histamine release around 20% in controls at that concentration. It is important to indicate here that in this group of control subjects, the histamine release was significantly lower than in the group of patients with a positive nasal culture against *S. aureus*. Furthermore, the culture-positive patients

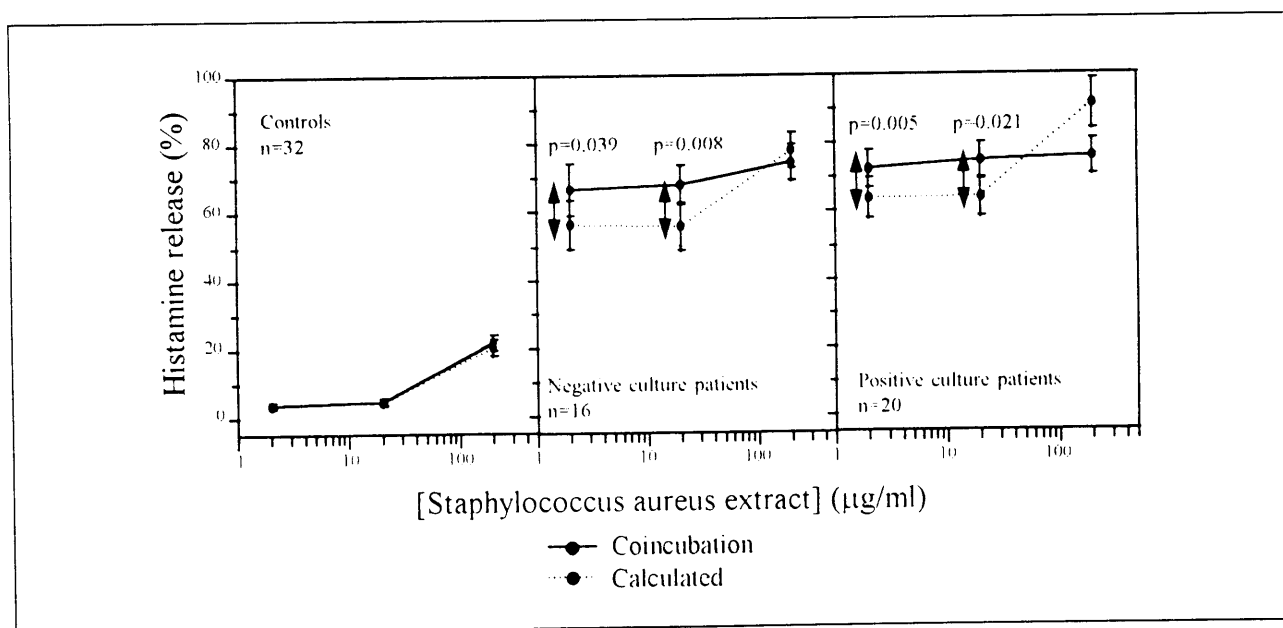


Fig. 6. Potentiation by *S. aureus* of histamine release induced by *D. pteronyssinus*.

showed a higher histamine release response to anti-IgE antibodies than the controls (Fig. 6), and a positive correlation was found between the response to *S. aureus* and anti-IgE. It is therefore possible that the patients with positive nasal culture against *S. aureus* were sensitized to the bacterium.

Regarding histamine release against the mite *D. pteronyssinus* in the presence of *S. aureus* (Figs. 3 and 4), histamine release is extremely important in this group, and as to be expected it was in agreement with the positivity obtained in the intradermal skin test and antigen-specific IgE determination, with a mean of 52.7% in patients with negative nasal culture, which increased up to 59.8% in patients with positive nasal culture. This discrete difference can be due to the fact that in patients with positive nasal culture, the infection can induce a greater hyperreactivity.

Of great importance is the histamine release obtained when the cells were incubated with *D. pteronyssinus* extract together with *S. aureus*. This cocubation caused a synergistic effect, i.e., a histamine release response exceeding the calculated sum of histamine release induced by *D. pteronyssinus* and *S. aureus* separately. The potentiation was found with lower concentrations of *S. aureus*, which in itself caused little histamine release, but is hardly obtained in higher concentrations. This is in agreement with other experiments on potentiation, demonstrating potentiation of a low response, but not of a high response. The potentiation we found is in accordance with results obtained by other authors examining histamine release induced by *D. pteronyssinus* or birch pollen in the presence of either *S. aureus* or lipopolysaccharide (LPS) of *Salmonella typhimurium* (8). This potentiation can be inhibited by means of some carbohydrates, such as galactose and *N*-acetylglucosamine, which indicates that sugar residues on bacteria or endotoxins are responsible for the potentiation by interacting with binding sites (possibly lectins) on the basophil cell membrane (6, 8). The potentiation obtained *in vitro* could represent an important mechanism in the aggravation of asthma during infections of the respiratory tract. Nevertheless, in some cases an IgE-dependent mechanism coexists, given that the elimination of the immunoglobulins from the basophil surface decreases or completely eliminates this histamine release in cells from some patients (18).

Considering the results obtained in this study, and as we reported in other articles (4, 19), the importance of bacterial infection in the etiopathogenesis of rhinosinus-bronchial hyperreactivity is confirmed. This fact has also been studied by several authors, given on the one hand the importance of the bacterial antigen IgE (11), and on the other hand, the increase in eosinophil cationic protein (ECP), very important in the bacterial infection, and scarce in the viral infection (20). This means that the immunopathological inflammatory mechanism set into motion in bacterial infections, where eosinophils play an

extremely important role, is much more important than in viral infections. In studies performed by us, we were able to prove that this viral infection had an immunological impact in the presence of specific antibodies in only 13.5% of the cases studied (21). This does not mean that in these patients the possibility of a viral infection is not considered; it is in many cases the first infection which will later give way to a bacterial infection.

Therefore, our results combined with those of Norn *et al.* confirm that the bacterial infection factor is very important in the etiopathogenesis of bronchial asthma, and especially in the treatment of infection. Again, we wish to emphasize the need to break the vicious circle of infection-inflammation-asthmatic response-epithelial flaking, which leads to further infection, and so on.

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RESUMEN

En la etiopatogenia del asma bronquial existen cada vez más evidencias del papel que desempeña la infección bacteriana, favoreciendo la inflamación y la obstrucción, es decir, el desencadenamiento de una respuesta asmática. Reunimos a 36 pacientes diagnosticados de asma bronquial con sensibilización a Dermatophagoides pteronyssinus, y a 32 sujetos sanos. Realizamos tests de liberación de histamina frente al extracto de Staphylococcus aureus solo o asociado a D. pteronyssinus y contrastamos su resultado con la presencia o no de S. aureus en el cultivo de secreción nasal. Encontramos liberación de histamina frente a S. aureus mayor del 10% únicamente a la concentración máxima (200 µg/ml), y resultó significativamente mayor en aquellos pacientes con cultivo nasal positivo. Con respecto a la liberación de histamina frente a D. pteronyssinus en presencia de S. aureus, hallamos una liberación por incubación significativamente superior a la resultante de la suma de la liberación frente a S. aureus y la liberación frente a D. pteronyssinus, tanto en pacientes con cultivo nasal negativo como positivo, en las concentraciones de 20 y 2 µg/ml. En conclusión, observamos una potenciación en la liberación de histamina frente a D. pteronyssinus con extracto de S. aureus en pacientes diagnosticados de asma bronquial. Estos hallazgos corroboran el papel importante que desempeña la infección bacteriana en la etiopatogenia del asma bronquial, y la importancia de un tratamiento dirigido frente a dicha infección.

Palabras clave: Liberación de histamina - Antígenos bacterianos - Potenciación de antígenos bacterianos - Asma bronquial - *S. aureus*

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