Original Article

Skin reactivity to autologous bacteria isolated from respiratory tract of patients with obstructive pulmonary disease

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Abstract: Bacterial flora of various strains was isolated from sputum and in some cases from BAL fluid of 75 patients with obstructive pulmonary disease experiencing dyspnea symptoms accompanying infections of respiratory tract. Among strains recognized traditionally as pathogenic in respiratory tract, we also isolated various strains typically called "normal oropharyngeal flora", "physiologic" or "non-pathogenic" bacteria. Those latter strains used in the skin tests in autologous manner for each patient had the property of provoking early (15 min) and late (24-48 h) reactions. Early reactivity resembles that induced in the tests with airborn allergens. This suggests a potentially important role of those currently ignored strains in pathogenesis of obstructive pulmonary disease.

Key words: Obstructive pulmonary disease, pathogenic flora, physiologic flora, skin tests.

Introduction

In several publications Norn and his group provided evidence that bacteria have the ability to induce histamine release or to enhance the inflammatory mediator release [1-4]. Such biological effects depend on two major mechanisms, i.e. IgE-mediated and non IgE-mediated reactions. The latter includes carbohydrate-lectin mediated reactions [3].

Our own observations concerning skin reactivity of the patients with obstructive pulmonary disease (qualified either as COPD or asthma) to autologous bacteria isolated from their respiratory tract showed that the bacteria used in the skin tests can provoke reactions of the immediate (15 min.), delayed (8-10 h) and late type (24-48h from introduction of the skin test). We found that different strains can provoke either all three types of reactions, two of them or just one, depending on the species of bacteria analyzed and on the individual patient's reactivity [5].

Bacteria growing extensively in the mucous secretions filling the respiratory tract of the patients with obstructive pulmonary disease are very difficult to eradicate with antibiotics [6-8] . These bacteria, that act on the patients respiratory tract not only by the mean of toxins and enzymes released, but also through induction of the allergic and para-allergic reactivity, need to be more thoroughly investigated. This study has been designed to show skin test reactivity to both pathogenic and nonpathogenic autologous bacteria isolated from the respiratory tract of individual patients.

Materials and methods

Patients

We studied 75 patients chronically suffering from obstructive pulmonary disease ranging between 18 and 65 years of age with the average age of 48 years. The duration of the disease ranged between 3 and 20 years; the average duration was 8 years. All of the patients were hospitalized, most of them repeatedly, because of the marked increase in dyspnea associated with signs and symptoms of infection. All patients were included in the trial and tested during infectious exacerbation of the

disease. Such exacerbations were diagnosed mainly based on the presence of: increased dyspnea, productive cough with muco-purulent sputum, increased sputum volume, increased sputum purulence, fever, malaise. In the course of their disease patients received typical treatment including bronchodilatating and anti-inflammatory drugs and antibiotics. During the period of the trial and at least for 2 weeks prior to their introduction to it, patients did not receive systemic glucocorticoids. In the medical records of most of the patients, the obstructive pulmonary disease was associated with diagnoses varying over the course of the disease. Diagnoses were also depending on the medical facility patients were diagnosed at. First clinical diagnosis was usually spastic bronchitis followed by the diagnosis of asthma or COPD. Immediately before the trial all patients included were diagnosed as suffering from COPD. The diagnosis was based on American standards [9].

Tested material

- Sputum
- Throat and nasal swabs
- BAL (broncho-alveolar lavage) in 1/3 of patients

Tested material, except for BAL, was obtained repeatedly (2-3 times) from each patient during disease exacerbation, and cultured in the routine media for aerobic and anaerobic bacteria. If patient received antibiotics, bacteriological tests were performed at least two weeks after withdrawal of such treatment.

Skin tests

We have tested skin reactivity of each patient to autologous bacteria. Each cultured bacterial strain was separated, killed with formaldehyde and suspended in saline at the concentration of $3x10^5$ cells per ml. Skin tests were performed by injecting 0.05 ml of bacterial suspension intradermaly on the forearm. As a positive control we used 0.05 ml of 0.1% histamine and the negative control was the injection of 0.05 ml of saline solution. Early skin reactivity was assessed after 15 minutes and the late one after 24 hours.

Early skin reactivity was assessed based on the diameter of the wheal and flare independently. As a positive result in early skin reactivity we accepted the wheal and flare measuring of at least half the size of positive control, assumed that negative control showed no reaction. As a strong reaction we accepted the wheal and flare of the size at least as large as provoked by histamine.

For the late type of reaction only flare was measured. The criteria for positive reaction of the late type were: positive – diameter no less then 5 mm., strong positive – diameter no less than 10 mm.

Results

As we have shown in our results (Table 1) a variety of bacteria species, able to provoke some degree of skin reactivity in autologous tests, can be found in

Table 1. Prevalence of bacterial strains provoking any type of autologous skin test reactions found in total 75 patients. Each strain provoked positive skin tests of either early, late or both types of reactions.

Pathogenic bacteria strains	**% (number of patients)**
Staphylococcus coagulase (+) positive	45.3 (34)
Moraxella catarrhalis	26.7 (20)
Haemophilus influenzae	17.3 (13)
Streptococcus pneumoniae	12.0 (9)
Haemophilus parainfluenzae	10.7 (8)
Klebsiella pneumoniae	2.7 (2)
Physiologic (nonpathogenic) bacteria strains	**% (number of patients)**
Streptococcus viridans	74.7 (56)
Neisseria species	56.0 (42)
Staphylococcus coagulase (-) negative	17.3 (13)
Streptococcus nonhemoliticus	17.3 (13)
Streptococcus beta-hemoliticus type B	10.7 (8)
Escherichia coli	9.3 (7)
Corynebacterium species	6.7 (5)
Candida albicans	2.7 (2)
Proteus vulgaris	1.3 (1)
Pseudomonas aeruginosa	1.3 (1)

Table 2. Typical reactivity to own bacterial strains cultured from respiratory tract of the 50 year old patient suffering from dyspnea for 12 years, repeatedly hospitalized in the past.

Pathogenic strains of bacteria	**Early reactions**	**Late reactions**
Moraxella catarrhalis	10/20	20
Staphylococcus aureus	10-25	5
Physiologic (nonpathogenic) strains of bacteria		
Streptococcus viridans	15/25	10
Staphylococcus epidermidis (coagulase negative)	7/25	10
Neisseria species	6/12	20
Controls		
Saline	0/0	0
Histamine	7/25	0

x/ – wheal size in mm.
/y – flare size in mm.

the respiratory tract of the patients with obstructive pulmonary disease. Usually several different species can be isolated from one patient. Some of them can be found more commonly than others. An example is given in table 2 showing the skin reactivity to autologous bacteria isolated from the respiratory tract of a patient with obstructive pulmonary disease. Most common bacteria belonging to a group usually called nonpathogenic, normal or physiologic bacteria in the respiratory tract were: *Streptococcus viridans* and *Neisseria species*. Other nonpathogenic bacteria found in a number of patients were: *Staphylococcus coagulase* negative, *Streptococcus nonhemoliticus, Streptococcus b-hemoliticus type B* and *Escherichia coli*. Among pathogenic bacteria *Staphylococcus aureus* and *Haemophilus influenzae* were the most prevalent, whereas *Streptococcus pneumoniae* were found rarely, and *Klebsiella pneumoniae* sporadically.

Nonpathogenic bacteria most frequently provoking strong early skin test reactivity were: *Staphylococcus coagulase* negative, *Neisseria species* and *Streptococcus viridans* (Table 3). The same strains gave most frequently strong positive reactions of the late type (Table 4). Among

Table 3. Prevalence of bacterial strains provoking strong autologous positive skin tests reactions of the early type in 75 patients. Reactions were measured 15 min. from the introduction of the test material.

Pathogenic strains of bacteria	**% (number of patients)**
Staphylococcus coagulase (+) positive	21.3 (16)
Haemophilus influenzae	5.3 (4)
Streptococcus pneumoniae	5.3 (4)
Haemophilus parainfluenzae	4.0 (3)
Moraxella catarrhalis	2.7 (2)
Physiologic (nonpathogenic) strains of bacteria	**% (number of patients)**
Staphylococcus coagulase (-) negative	20.0 (15)
Neisseria species	13.3 (10)
Streptococcus viridans	10.7 (8)
Escherichia coli	6.7 (5)
Streptococcus nonhemoliticus	4.0 (3)
Corynebacterium species	2.7 (2)
Candida albicans	2.7 (2)
Pseudomonas aeruginosa	2.7 (2)
Streptococcus beta-hemoliticus type B	1.3 (1)

Table 4. Prevalence of bacterial strains provoking strong autologous positive skin tests reactions of the late type in 75 patients. Reactions were measured 24-48 h from introduction of the test material.

Pathogenic strains of bacteria	**% (number of patients)**
Staphylococcus coagulase (+) positive	13.3 (10)
Haemophilus influenzae	8.0 (6)
Moraxella catarrhalis	8.0 (6)
Streptococcus pneumoniae	5.3 (4)
Haemophilus parainfluenzae	4.0 (3)
Physiologic (nonpathogenic) strains of bacteria	**% (number of patients)**
Staphylococcus coagulase (-) negative	17.3 (13)
Neisseria species	10.7 (8)
Streptococcus viridans	10.7 (8)
Escherichia coli	6.7 (5)
Streptococcus nonhemoliticus	4.0 (3)
Streptococcus beta-hemoliticus type B	2.7 (2)
Corynebacterium species	2.7 (2)
Proteus vulgaris	1.3 (1)
Pseudomonas aeruginosa	1.3 (1)

pathogenic bacteria strong early skin test positive reactions were provoked mainly by *Staphylococcus aureus* and less commonly by: *Haemophilus influenzae, Streptococcus pneumoniae, Haemophilus parainfluenzae* and *Moraxella catarhalis*. Similar pattern among these bacteria is characteristic for the positive skin tests results of a late type except for more common strong late reactivity with *Moraxella catarrhalis* strains.
Most patients responded to their own bacteria with both early and late skin test reactions. Only in some cases we observed either early or late reaction.

Discussion

In physiological conditions the lower respiratory tract is free of bacteria. Bacteria can be found there as a result of acute infection with bacterial strains of: *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and increasingly common in the recent years: *Chlamydia, Mycoplasma* and *Legionella*. Bacterial presence in the lower respiratory tract can also be secondary to viral infections that damage ciliated bronchial epithelium and increase mucus production as a result of inflammation. Excessively secreted mucus may become a "hideout" and feeding medium for bacteria aspirated to the bronchial tree.
Regardless of the mechanism of introduction, bacterial presence in the lower respiratory tract is generally related to some kind of inflammatory response. The airways of smokers seem to be the exception, because normal oropharyngeal flora can be found there in asymptomatic smokers as well as in smokers with chronic bronchitis [10]. Bacteria isolated from lower respiratory tract belong not only to the group of commonly recognized "pathogens". Also so called "physiological" or "normal" strains can inhabit the bronchial tree using mucus as a growth medium. Such strains belong mainly to: *Streptococcus viridans* and *Neisseria catarrhalis,* which is confirmed by our results. These "nonpathogenic" bacteria have a strong tendency to provoke early and late skin tests reactions, as it is also documented in this experiment. This indicates some kind of bacteria–induced mediator release, possibly involved also in the pathomechanisms in the bronchi. Acting in concert with infections caused by viruses and "classically" pathogenic bacteria, they may create a vicious circle of the respiratory tract inflammatory reactions (allergic, pseudoallergic and infectious) which could be hard to break. Management of such states is very difficult because the antibiotics administered can just reduce, but usually not eliminate the infection [7,8]. Furthermore, antibiotics can reach only those bacteria that grow directly on the surface of the mucus membrane or in the tissue, but fail to eradicate bacteria hidden in the excessively produced mucus. Concentration of antibiotics in the mucus cannot reach effective level.
Streptococci deserve separate analysis. Their common presence and the ability to provoke strong reactions of the early as well as the late types, point to the their possible role in pathogenesis of the obstructive pulmonary disease. Very interesting finding of basophil-bound IgE and serum IgE directed against *Streptococcus pneumoniae* in patients with chronic bronchitis during

acute exacerbations, also suggests non-classical pathomechanisms related to streptococcal infection [11]. Our trial findings, that *Streptococcus viridans*, but also *Neisseria* spp. and other "nonpathogenic" bacteria have the ability to provoke early and late autologous skin test reactions may suggest their involvement in pathogenesis of COPD with infectious exacerbations complicated by obstruction in the airways. On the other hand, isolating such flora not only in the course of chronic bronchitis in smokers but also in asymptomatic smokers, as shown by Ovarfordt [10], can suggest their lack of involvement in the pathomechanism of bronchial obstruction. One of the possible explanations of this contradiction may be derived from the outcome of our study. If we assume, based on our results, that "normal flora" can provoke allergic or pseudoallergic type of reactions, not only the presence of this bacteria in the bronchi may decide of the development of spastic symptoms. Also hyperreactivity to these species is needed, which depends on allergic or pseudoallergic reactions. Currently *Streptococcus viridans*, like other "nonpathogenic" bacteria, are not considered to be a factor leading to development of obstructive pulmonary disease [12, 13]. However, difficulties to verify the presence or absence of nonpathogenic species and subtypes in the airways may explain this opinion. It should be recalled that in the bacteriological laboratories, examination of e.g. *Streptococcus viridans* or *Neisseria* species is usually followed only by the statement: "physiological flora was found" or "no pathogenic bacteria were found". This contributes to the medical professions, including lung specialists attempting to explain the pathogenesis of COPD, to ignore the colonization of the bronchi by this bacteria.

The results presented herein should induce a more exhaustive analysis of the presence of so called "nonpathogenic" bacterial flora found in the lower respiratory tract of patients with obstructive pulmonary disease. Such analysis may lead to better explanation of the role of bacteria in bronchial asthma [14] and in a larger sense, in the obstructive lung disease [15]. This should also induce a thorough review of the value of vaccine use in asthma with symptom exacerbations during infections [5, 16].

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