

Institute for Public Health of Middleczech County,
Prague 2., Studničkova 7, Czechoslovakia.
Institute for Microbiology and Department of Medicine of University
of Pilsen, Czechoslovakia.

AUTOIMMUNE MECHANISM IN DISEASES OF THE RESPIRATORY TRACT

By

V. WAGNER, A. TOMŠÍKOVÁ, J. ŠACH, J. JANKOVÁ &
D. NOVÁČKOVÁ

Autoimmune diseases are much more frequent than authors previously believed (1). It seems that autoimmunization is a common biological principle as widespread as allergy (2, 20). It appears, however, that the autoantibodies may have not only an immunotoxic function, damaging the organism, but also a physiological one, removing denaturated tissue.

Bergquist (3) suggests that the autoimmune mechanism may be a secondary factor in addition to allergy in bronchial asthma. *Mendes* (4) succeeded in causing bronchial asthma in guinea pigs by applying an antigen from human lung tissue.

We were interested in the question of whether yeast-like organisms could also change some tissues into an antigen. Yeast-like organisms showed a stimulating influence on the production of skin autoantibodies (5, 6), as well as an increase of chronic bronchitis (7, 8) and bronchial asthma in our country (9) as well as in North Vietnam (10, 11) and in experimental bronchitis (12, 13).

METHODS

Groups of 166 individuals with chronic bronchitis, 23 individuals with bronchial asthma, 39 individuals suffering from silicosis and 100 healthy persons were examined.

In all persons the sputum and laryngeal swabs were investigated by cultivation on blood agar plates, Sabourad and Littmann agar plates and liver broth. According to the cultivation of the yeast-like fungi and staphylococci, each group of patients was divided into cases with a positive or negative finding of yeast-like organisms and staphylococci.

A collodion-agglutination using the method of *Cavelti* (14) modified by *Wagner* (15, 16) was carried out on sera to establish autoantibodies against lung, whole bronchi, bronchial mucous membrane, liver, heart and kidney. These antigens were prepared from the organs of deceased persons without pathological changes. The lung tissue was cleared of blood by repeated washing, the mucous membrane was removed from the wall of the bronchi. The tissue of all organs was minced and homogenized into pulp, mixed with B₂ solution (9,6 g NaCl, 0,4 g CaCl₂, 0,1 g NaHCO₃, 1000 ml ag. bidest.) with 0,02 per cent Merthiolate at a ratio of 1:5, frozen at -20° C for 24 hours, then centrifuged at 1500 r/min. per 10 minutes and stored at -20° C. The supernatant was coated on a basic suspension of collodion particles at a ratio of 1:20 for 1 hour. The final density of the mixture corresponded to No. 2-3 on the *Mac Farland* scale (17) and the final dilution of the tissue extract was 1:100.

Sera were diluted geometrically in a solution of B₂, 0,25 ml of each dilution was put in a test tube 0,8 in diameter. The same amount of antigen-coated particles was mixed with the serum. Particles without serum and particles with negative serum served as controls. After 1 hour at room temperature all tubes were centrifuged 5 min. at 1500 r/min. and the results read by the naked eye during gentle stirring at the bottom of the test tube. Clear-cut positive results were taken into account in the presence of clear negative results in the controls.

RESULTS

Fig. 1 shows the finding of autoantibodies against the investigated antigens in all examined groups. The autoantibodies against the control organs (heart, liver, kidney) in all groups were formed only in a small percentage of cases and in low titres. Likewise the autoantibodies against lung tissue were found only in some of the examined patients, especially in cases of chronic bronchitis, complicated by an affection of lung tissue. On the other hand the autoantibodies against bronchial mucous membrane and bronchi were found in great quantity in groups of bronchial asthma and chronic bronchitis. The presence of these antibodies investigated by means of the Pearson χ^2 test was statistically highly significant (0,01 per cent) in comparison with the group of silicosis patients and

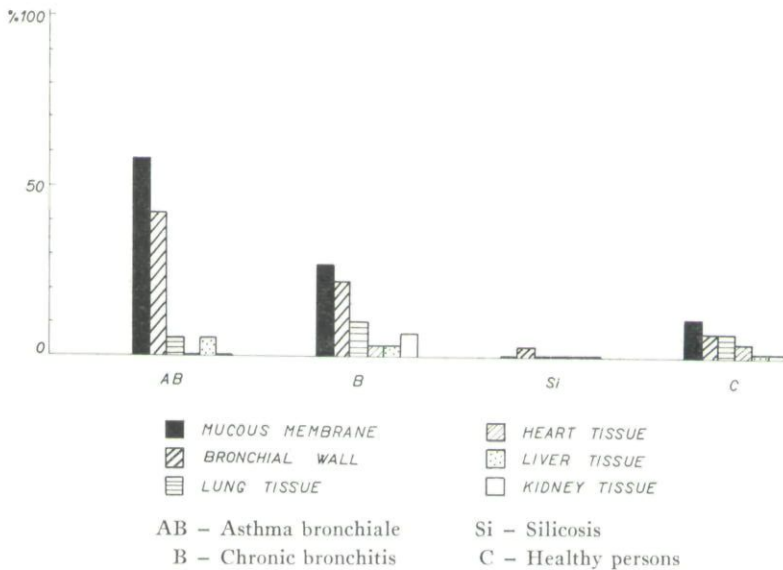


Fig. 1.

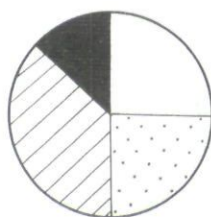
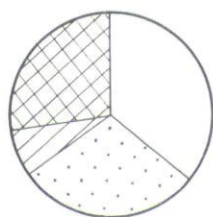
Finding of Autoantibodies in all Examined Groups.

healthy persons. The smallest percentage of autoantibodies against any organ was found in patients suffering from silicosis. This figure also shows, that the antigen prepared from the bronchial mucous membrane alone binds more antibodies than the antigen prepared from whole bronchi. Statistical comparison showed a significant presence of autoantibodies determining the antigen of the bronchial mucous membrane.

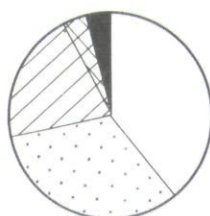
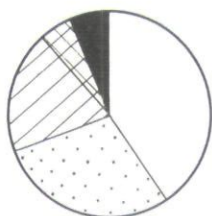
Fig. 2 shows incidence of the positive titers of autoantibodies against mucous membrane and bronchial wall. It is apparent that the autoantibodies reached a lower titer in the group of healthy persons in comparison with bronchial asthma and chronic bronchitis, where we found higher titers (1:128 or more).

Fig. 3 shows the finding of the autoantibodies against the mucous membrane and bronchi in cases with positive or negative culture of the yeast-like organisms and staphylococci in the respiratory tract. By means of the Pearson χ^2 test it was

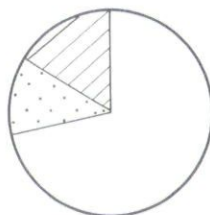
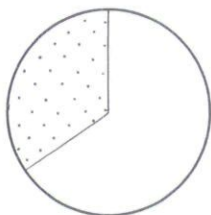
ASTHMA BRONCHIALE



CHRONIC BRONCHITIS



HEALTHY PERSONS



AUTOANTIBODIES AGAINST BRONCHIAL MUCOUS MEMBRANE

AUTOANTIBODIES AGAINST BRONCHIAL WALL

TITER

□ 1:8

◻ 1:16

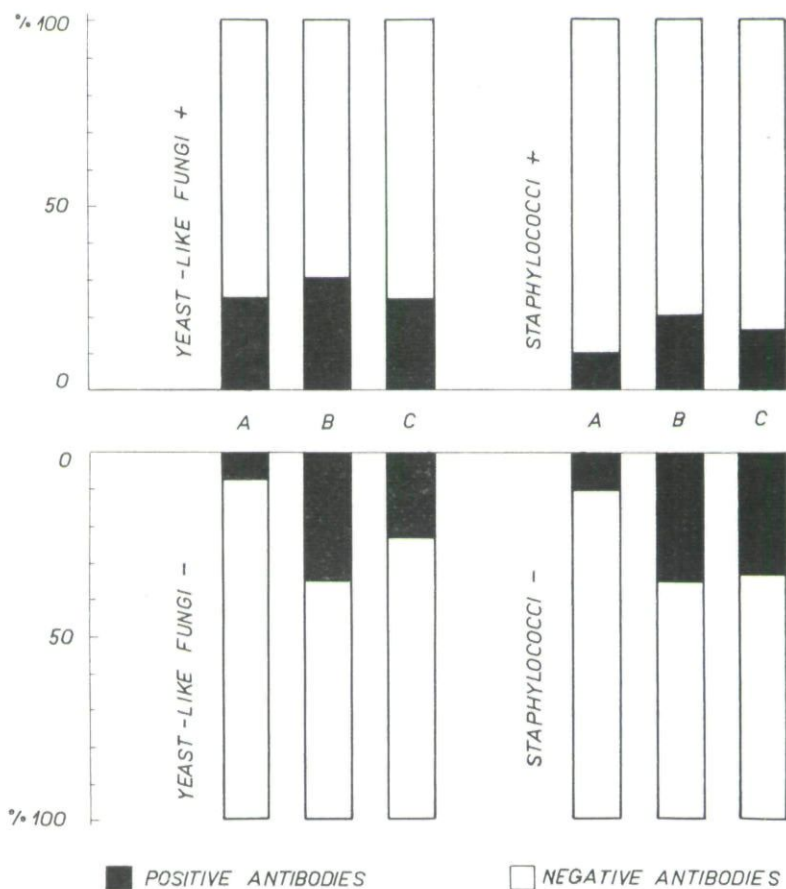
▨ 1:32

▩ 1:64

■ 1:128

Fig. 2.

Positive titers of antibodies against bronchial mucous membrane and bronchial wall.



A - Antibodies against lung tissue
 B - Antibodies against mucous membrane
 C - Antibodies against bronchi

Fig. 3.

Autoantibodies in cases with the positive and negative finding of yeast-like fungi and staphylococci in the respiratory tract.

proved that the presence of the autoantibodies is not related to the finding of the yeast-like fungi or staphylococci in the respiratory tract. An exception was found in the chronic bronchitis group, where the autoantibodies against bronchi are sig-

nificantly related to the finding of the yeast-like fungi in the respiratory tract (at the 5 per cent level of significance).

DISCUSSION

Our results place chronic inflammation of the respiratory tract among the diseases with an autoimmune antibody mechanism. This process occurs least of all in silicosis, more in chronic bronchitis (especially when the lung tissue has been affected) and in the greatest amount in bronchial asthma. Obviously in these diseases there act some factors: infection of external or internal origin, physical or chemical influences on the bronchial tissue which changes its antigenic structure so that it is recognized by immunological competent cells as "foreign". The same conclusion was reached by *Bergquist* (3). He used an extract of the medium containing tonsillar or nasal mucous membrane tissue, on which staphylococcus aureus had been cultivated, for the skin vaccination of the patients with bronchial asthma and of healthy persons. *Bergquist* (3) believes this microbe to be an important factor in the pathogenesis of bronchial asthma.

We could not, however, prove any relationship between the occurrence of staphylococcal and autoantibodies. Neither did the yeast-like fungi showed any relation to the autoimmunization except in chronic bronchitis, where there are a few more autoantibodies in the presence of the yeast-like fungi. Even though it is possible to object on the grounds that autoimmunization is a problem of immunological reactivity of individual (18, 19), our findings do not favour the hypothesis of participation of staphylococci and yeast-like organisms in the process of autoimmunization against the tissue antigens of the respiratory tract.

It is interesting that the positive findings of the autoantibodies reacting with the antigens of the respiratory tract in chronic bronchitis and bronchial asthma, are not followed by the occurrence of autoantibodies against the internal organs. This means that the autoimmunization in these cases remains

"superficial" because the lung tissue gives less often positive reaction for autoantibodies with the serum of these patients. The highest finding of autoantibodies was noticed against bronchial mucous membrane alone. In contradiction to *Mendes* (4) the lung tissue binded autoantibodies in our experiment only in a small number of cases.

Our findings do not necessarily prove that autoimmunization plays a part as pathogenical factor in a immunotoxic sense, or whether it is only a secondary phenomenon, accompanying a chronic disease as a tissue allergy of tuberculin type appears after chronic infections. A third possibility should be considered that the autoantibodies have a protective effect on the cells according to the observations of *Paterson* (21).

SUMMARY

A search was made for autoantibodies in individuals with chronic bronchitis, bronchial asthma, silicosis and in healthy persons by means of the collodion agglutination technique with tissue extract.

The finding of autoantibodies against bronchial mucous membrane was statistically highly significant in bronchial asthma and chronic bronchitis in comparison with the group of silicosis patients and healthy persons.

The incidence of autoantibodies against internal organs (heart, kidney, liver) was low in the groups of patients suffering from respiratory disorders. The smallest percentage of autoantibodies against any tissue was found in patients suffering from silicosis. The antigen prepared from the bronchial mucous membrane alone binds more antibodies than the antigen prepared from whole bronchi.

No correlation between the finding of autoantibodies and the presence of staphylococci or yeast-like fungi in the respiratory tract could be established.

REFERENCES

1. *Miescher, P. & Vorlaender, O.*: Immunopathologie in Klinik und Forschung und das Problem der Autoantikörper, Stuttgart, 1960.
2. *Wagner, V.*: Imunotoxická jako pathogenetický mechanismus nemoci, Vnitřní lék. 1/7, 481-490, 1955.
3. *Bergquist, G.*: Contribution to the Problem of Autosensitisation in Bronchial Asthma, Acta Allergol., X, 187-193, 1956.
4. *Mendes, E.*: Experimental Asthma of the Guinea Pig Provoked by Human Lung Tissue, Acta Allergol., XII, 231-39, 1958.
5. *Tomšíková, A., Wagner, V. & Blahníková, D.*: Tvorba kožních autoprotilátek vlivem synergického účinku homologního kožního antigenu a Kandid u krys., Čs. dermatologie, 34/5, 314-24, 1959.
6. *Wagner, V. & Tomšíková, A.*: Ein experimentaler Beitrag zu Autoimmunisierungsfähigkeit von Hefen mit Hautantigen bei Ratten, Zeitsch. Immunitätsforsch., 119, 270-278.
7. *Tomšíková, A., Šach, J., Hořejší, M., Mecl, A., Malý, V. & Nováčková, D.*: Vliv mykotické flory na vznik chronické bronchitidy, Čas. lék. čes., 45, 1339-49, 1962.
8. *Tomšíková, A., Šach, J., Hořejší, M., Mecl, A., Malý, V. & Nováčková, D.*: Mykologische Studien bei der chronischen Bronchitis. Z. für die gesamte innere Med. 17/18, 872-78, 1962.
9. *Tomšíková, A., Zavázal, V., Malý, V., Nováčková, D. & Nová, H.*: Vztah kvasinek a plísní k astmatu bronchiálnímu v ČSSR, in print.
- 9 a. *Tomšíková, A., Zavázal, V., Nováčková, D. & Nová, H.*: Mykologische Studien bei den Respiratoriumsallergien, in print.
10. *Černík, L., Doan hong-Hoa, Vu dinh-Hai, Tomšíková, A., Pham van-Nong & Výmola, F.*: Über den Einfluss der mykotischen Flora auf Bronchialasthma in Tropen, Allergie und Asthma, 8, 132-144, 1962.
11. *Černík, L., Doan hong-Hoa, Vu dinh-Hai, Tomšíková, A., Pham van-Nong & Výmola, F.*: Vplyv mykotické flóry na bronchiálnu astmu v tropických podmienkách, Čas. lék. čes. CII/48, 1313-22, 1963.
12. *Tomšíková, A., Důra, J. & Nováčková, D.*: Ein experimentaler Beitrag zur chronischen Bronchitis bei Kaninchen. Z. für die gesamte innere Med. 19/15, 613-17, 1964.
13. *Tomšíková, A., Důra, J., Malý, V. & Nováčková, D.*: Vliv mykotické flory na vznik chronické bronchitidy. III. Experimentální část, in print.
14. *Cavelti, P. A.*: Collodion Agglutination, J. Immunol., 157, 141-148, 1947.
15. *Wagner, V.*: Der Einfluss der Elektrolyten auf die Kolodium-Agglutination Schweiz. Zeit. Allg. Pathol. Bakter. 17, 94, 1954.
16. *Wagner, V., Rejholec, V., Mandlíková, Z. & Křížová, M.*: Kolodiová aglutinace, Čs. biologie, 1/6, 376-382, 1952.

17. *McFarland, J.*: The Nephelometer, an Instrument for Estimating the Number of Bacteria in Suspensions used for Calculating the Opsonic Index and for Vaccines. *JAMA*, 40, 1176-78, 1907.
18. *Rejholec, V., Fenc, V., Repis-Schlechtá & Hellerová, E.*: Reaktivita imunologica nello stato dis alute ed in certe situazioni patologiche, reattivita di pazienti afetti da glomerulonefrite dopo una semplice inoculazione antigenica di *Brucella abortus*, studiata a mezzo del titolo delle agglutinine e degli anticorpi incompleti. *Minerva nefrolog.*, 2, 62/64, 1955.
19. *Wagner, V., Rejholec, V. & Malý, V.*: Agglutination and incomplete Antibodies after a Single Antigenic Inoculation in Normal and Rheumatic Individuals, *Ann. Rheum. Dis.*, 14, 243-250, 1955.
20. *Wagner, V.*: Imunotoxické nemoci, *Čas. lék. českých*, 88, 981-4, 1949.
21. *Paterson, P. Y.*: Cells, Antibodies and Autoimmune Disease. In *Monography: Cell-bound Antibodies* p. VI, Wistar Press, Philadelphia, 1963.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.