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ALLERGENIC PROPERTIES OF TRICHOMONADS INHABITING HUMAN RESPIRATORY TRACT

Keeping within the limits of the complex investigation at our department, during which the detection of trichomonads in the respiratory tract of patients with different chronic pulmonary diseases (Терас et al., 1980) as well as that of antibodies specific of trichomonads in the blood sera of the same patients was studied (Казакова et al., 1980; Рыйгас et al., 1980), we tried to clear up whether these protozoa have also allergenic properties and whether specific allergy is detectable in patients with trichomonads in the bronchi. Simultaneously such studies represent an additional part in the complex investigation of the biological properties of all trichomonads inhabiting human organism, while previously the allergenic properties of trichomonads isolated from the genito-urinary tract were demonstrated at our department (Яакмээс, 1964; Терас, 1973). The specific allergy in patients suffering from genito-urinary trichomoniasis has also been investigated (Терас et al., 1965, 1976; Jaakmees, Teras, 1966; Teras, 1973).

Material and methods

To solve the tasks of the given investigation, first of all we made series of experiments on experimental animals sensitized with trichomonads isolated both from the human respiratory tract and oral cavity. For our experiments we used guinea-pigs because these animals are considered the best test objects for studying the allergenic properties both of microorganisms and their preparations (Беклемишев, 1968; Вершигора, 1971). All together 8 axenic strains of *T. tenax* and 61 guinea-pigs were used. For axenisation and cultivation of the strains of *T. tenax* we used the methods and the TT-1 medium elaborated at our department earlier (Терас et al., 1970).

The guinea-pigs were sensitized intraperitoneally, subcutaneously and intracutaneously with axenic cultures of those strains. When sensitized intraperitoneally, every experimental animal was injected into the abdominal cavity with 50 mln live protozoa that were grown in TT-4 medium (Терас et al., 1970) and washed thrice in saline by means of centrifugation. For subcutaneous method each animal was injected with 50 mln protozoa 4 times at 7-day intervals. During intracutaneous sensitization the guinea-pigs were injected with 5 mln protozoa 5 times at 7-day intervals.

For intraperitoneal sensitization of the guinea-pigs we used the cultures of serotype strains A, B, C and D of *T. tenax*, isolated from the oral cavity and identified earlier at our department (Терас et al., 1972; Кумм et al., 1973). Subcutaneous and intracutaneous sensitizations were carried out with the cultures of serotype strain C of *T. tenax* and four strains of trichomonads isolated from the patients suffering from pul-

monary diseases, one strain (53 I) being isolated from the oral cavity, another one (206 II) from sputum and two (53 III and 206 III) from the bronchi.

The results of the sensitizations were tested by means of intracutaneous injections of corpuscular allergens prepared from the cultures of the same strains used for the sensitization of experimental animals. In addition, the intraperitoneally sensitized guinea-pigs were tested for obtaining comparative data with allergens prepared from the serotype strain TN of *Trichomonas vaginalis* and strain II of *Pentatrichomonas hominis*, identified at our department by J. Teras (Tepac, 1963) and I. Kazakova (Казакова, 1975).

In order to prepare corpuscular allergens we used the methods worked out at the investigation of the diagnostic value of the intradermal test in cases of genito-urinary trichomoniasis (Яакмээс, 1964; Jaakmees, Teras, 1966). In our investigation we used the 48-hour axenic cultures of trichomonads grown in TT-4 medium (Tepac et al., 1970) for preparing the corpuscular allergen. Having thrice washed the cultures in saline by means of centrifugation, we stated their density and then prepared a suspension containing 2.5 mln trichomonads in 1 ml of saline to which we added 0.5 per cent of phenol. After testing its sterility, the allergen was put into ampules (1.0 ml in every ampule) and stored at 4° before use.

We carried out the intracutaneous tests on the lateral sides of the guinea-pigs' stomachs. Having removed the hair and disinfected the skin of the animals, we injected them intracutaneously with 0.1 ml of each corpuscular allergen and for control — with 0.1 ml of saline with an addition of 0.5 per cent phenol. The results of such a test were studied 24 and 48 h after the injections, considering the skin reaction with a diameter of 4 mm and more to be positive and that of a smaller diameter — negative.

Having obtained experimental data on the allergenic properties of trichomonads inhabiting the human respiratory tract, we carried out an intracutaneous test with corpuscular allergen in 30 patients with different pulmonary diseases out of the contingent examined by us in order to detect trichomonads in the bronchi, sputum, oral cavity, intestinal and genito-urinary tracts. Among these 30 patients, 16 had trichomonads in the bronchi. At that in 6 cases trichomonads were found simultaneously in the oral cavity and bronchi, in one person — in the sputum and bronchi. In the remaining 14 patients we stated an absence of trichomonads in the oral cavity as well as in the respiratory, genito-urinary and intestinal tracts.

For carrying out the intracutaneous test, corpuscular allergens prepared as described above were used. The technique of the intracutaneous test on human beings was realized as follows: having disinfected the lateral side of the shoulder with 70 per cent ethanol, we injected intracutaneously 0.1 ml of each allergen and for control — 0.1 ml of saline with an addition of 0.5 per cent phenol.

The intracutaneous tests were carried out with corpuscular allergens of 5 strains of trichomonads simultaneously. Two of these strains were isolated from the oral cavity (serotype strain C and 53 I), another one (206 II) from sputum and two (53 III and 206 III) from bronchi. The distances between the points of allergen injections were at least 5 cm. The results of the tests were evaluated after 24 h according to the size of nodules, taking into consideration that those with a diameter of 6 mm and more were positive (+) and those with a diameter of less than 6 mm — negative (-).

Results and discussion

According to the results obtained we established the existence of allergy to *T. tenax* in the experimental animals already in two weeks after their intraperitoneal injection with axenic cultures of serotype strains of trichomonads isolated from the oral cavity.

As shown in Table 1, all the four serotype strains of *T. tenax* (A, B, C and D) revealed allergenic properties. So, the allergic reaction to the allergen of the serotype strain used for sensitization was positive in all cases. Nevertheless, the reaction was negative in some animals sensitized with allergens of the remaining three heterologous serotype strains of *T. tenax*. For example, the intracutaneous test with allergens of serotype strains A, C and D was not positive in all the guinea-pigs sensitized with allergens of serotype strain B of *T. tenax*, though allergy to the allergen of the homologous strain was stated in all these animals.

The distinctions turned out to be more contrasting when the allergens used for intracutaneous test were prepared from the strains of other trichomonad species. Thus the tests with allergens both of serotype strain TN of *T. vaginalis* and serotype strain II of *P. hominis* were negative in all the guinea-pigs sensitized intraperitoneally with serotype strains of *T. tenax* (Table 1). This shows clearly that the allergy induced by *T. tenax* is species-specific.

Table 1

Data on sensitization of guinea-pigs by means of intraperitoneal injection of *T. tenax* cultures

Strain used for sensitization	Number of experimental animals	Number of animals with positive intracutaneous test obtained with allergens of the serotype strain						Control with saline
		<i>T. tenax</i>				<i>T. vaginalis</i> TN	<i>T. hominis</i> II	
		A	B	C	D			
A	5	5	5	4	4	0	0	0
B	5	1	5	3	1	0	0	0
C	6	5	3	6	3	0	0	0
D	4	1	3	3	4	0	0	0

Table 2

Data on sensitization of guinea-pigs by means of intracutaneous and subcutaneous injections of *T. tenax* cultures

Strain used for sensitization	Method of sensitization	Number of experimental animals	Number of animals with positive intracutaneous test obtained with allergens of the strain					Control with saline
			Serotype C	53 I	53 III	206 II	206 III	
Serotype C								
C	Intracutaneous	4	4	4	2	3	2	0
53 I	"	4	2	3	3	3	3	0
53 III	"	4	3	4	3	3	2	0
206 III	"	4	2	1	4	4	4	0
Serotype C								
C	Subcutaneous	5	5	5	3	5	3	0
53 I	"	5	3	5	5	4	3	0
53 III	"	5	3	5	5	4	5	0
206 II	"	5	4	4	5	5	5	0
206 III	"	5	4	5	4	4	5	0

Having obtained preliminary data on the allergenic properties of the serotype strains of *T. tenax* we went on with our experiments using for the sensitization of the guinea-pigs the strains of trichomonads isolated from both the oral cavity (strain 53 I and serotype strain C) as well as from the respiratory tract (strains 53 III, 206 II and 206 III) of patients with pulmonary diseases. In these cases, unlikely to the previous experiments, the guinea-pigs were sensitized not intraperitoneally, but intracutaneously and subcutaneously.

Analysing the results obtained by intracutaneous tests on the animals sensitized intracutaneously or subcutaneously (Table 2) we found that, like serotype strains of *T. tenax*, the strains from the bronchi, sputum and the oral cavity of patients with pulmonary diseases and used in the given experiments had also allergenic properties. Nevertheless, it should be noted that the skin reaction with the allergen of the homologous strain was almost always positive, but sometimes absent with the allergens of other strains.

In our experiments the sensitization of guinea-pigs by means of the subcutaneous method occurred to be somewhat better in comparison with the intracutaneous technique. So, the intradermal test with the homologous allergen was positive in all experimental animals sensitized subcutaneously, but not in all those sensitized intracutaneously. Thus we found that the results of intracutaneous tests on the experimental animals sensitized with various strains of *T. tenax* depended to a great extent on the strain used for preparing the allergens and on the method of sensitization.

Having found that the serotype strains of *T. tenax* as well as the trichomonads isolated from the bronchi and sputum of patients with pulmonary diseases had allergenic properties, after a thorough examination of sterility of all the used allergens and control solutions, we carried out intracutaneous tests on 30 patients with pulmonary diseases. We used five corpuscular allergens prepared from axenic cultures of serotype strain C and of strains 53 I, 53 III, 206 II and 206 III of *T. tenax*.

As mentioned above, in our preliminary investigations we stated the absence of trichomonads in the oral cavity, respiratory, genito-urinary and intestinal tracts of 14 patients. Thus we may consider them belonging to the control group. The obtained data showed that intracutaneous tests with all five allergens occurred to be negative in all these 14 patients. But the results revealed by the intracutaneous test in the patients with trichomonads in the bronchi were quite different (Table 3). We found that 24 h after an intracutaneous injection of corpuscular allergens there was not only a clearly expressed hyperemia at the point of injection of at least one of the allergens but also a nodule with a diameter not less than 6 mm. Four persons (patients 1, 7, 11, 16) had such reaction only with one allergen, 10 persons (patients 2, 3, 5, 6, 8, 9, 10, 12, 14, 15) with 2 allergens and 2 persons (patients 4, 13) with 3 allergens. The greatest number of positive reactions was obtained with allergens made of the cultures of the strains 53 I (from the oral cavity) and 206 III (from the bronchi). Both of them caused an allergic reaction in 8 patients, but reactions with both allergens occurred to be simultaneously positive only in four of the investigated persons.

Thus we found that the results of intracutaneous tests on patients with pulmonary diseases in whom trichomonads were detected in the respiratory tract depended to a great extent on the strain used for preparing the allergen. In connection with it not a single allergen alone proved suitable for the detection of allergy in all the patients under

examination. Consequently it is necessary to use several different allergens simultaneously.

It is worth mentioning that in the blood sera of the same patients with trichomonads in the respiratory organs specific antibodies could be detected: specific agglutinins for *T. tenax* in titres of $\geq 1:320$ (Казакова et al., 1980) and specific complement fixing antibodies — $\geq 1:40$ (Рыйгас et al., 1980). The data obtained by the agglutination reaction and complement fixation as compared with the results of the allergic test are presented in Table 3. We have taken into consideration only the presence (+) or absence (-) of specific antibodies. Comparing the results of these reactions, we have revealed that sometimes the intracutaneous test was positive with the allergen of such a strain by means of whose antigens the results of the agglutination reaction and complement fixation are negative. In the blood sera of the same patients the specific antibodies could be found by using the antigens of another strain (patients 1, 3, 7, 9 a. o.). Such results depend on the fact that the trichomonad strains have various antigenic properties and are classified in serotypes (Кумм et al., 1973). Nevertheless, in spite of the fact that the results of the agglutination reaction and complement fixation depended on the strain used for preparing the antigen, we could not state any correlation between the results of these reactions and the intracutaneous test. But it is obvious that all the data obtained by these investigations have complemented each other.

Thus, the results of the given investigation occurred to be analogous with the results obtained at our department by intracutaneous tests on patients with genito-urinary trichomoniasis (Яакмээс, 1964; Jaakmees, Teras, 1966; Терас et al., 1976), since this research also showed that the application of only one allergen prepared of one strain of *T. vaginalis* was insufficient for diagnostic purposes. But in the same investigations the intracutaneous test was considered as a highly valuable diagnostic method for genito-urinary trichomoniasis of men and women by simultaneous application of allergens prepared from various serotypes of these protozoa.

Summing up the data obtained during the given investigation it is possible to say that trichomonads isolated from the human respiratory tract have allergenic properties and can induce specific allergy both in experimental animals sensitized with these protozoa and in humans with trichomonads in the bronchi. Such an allergy is detectable by the intracutaneous injection of specific corpuscular allergens. For carrying out these investigations it is necessary to use simultaneously more than one strain for preparing antigens from axenic cultures of the trichomonads inhabiting the respiratory tract and oral cavity of man.

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Received
July 7, 1982

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INIMESE HINGAMISTEEDES ELUTSEVATE TRIHHOMOONASTE ALLERGEENSETEST OMADUSTEST

Inimese hingamisteedes elutsevate trihhomoonaste allergeensete omaduste uurimiseks tehti eelkõige katsed merisigadega, keda sensibiliseeriti intraperitoneaalselt, subkutaanselt ja intrakutaanselt kopsuhaigusi põdevate inimeste bronhidest ja suuõõnest isoleeritud trihhomoonaste akseeniliste kultuuridega. Reaktsiooni tulemusi kontrolliti samadest kultuuridest valmistatud korpuskulaarsete allergeenide nahasisese manustamise teel. Ilmnes, et allergeensed omadused esinesid nii hingamisteedest kui ka suuõõnest isoleeritud trihhomoonaste tüvedel, kusjuures sensibiliseerimiseks kasutatud tüvest valmistatud allergeeniga testimisel oli reaktsioon tugevam kui teiste tüvede allergeenide puhul. Seejuures tuleb märkida, et *T. tenax*'i tüvedega sensibiliseeritud merisigadel ei esinenud allergilist reaktsiooni *T. vaginalis*'e serotüüpilise TN ning *P. hominis*'e II serotüüpilise suhtes.

Pärast katseandmete saamist, mis kinnitasid allergeensete omaduste esinemist *T. tenax*'i kõigil uuritud tüvedel, tehti intradermaaltset viie korpuskulaarse allergeeniga 30-le kopsuhaigusi põdevale inimesele. Kasutatud allergeenidest olid kaks valmistatud bronhidest isoleeritud trihhomoonaste tüvedest, üks rögest ning kaks suuõõnest isoleeritud tüvedest. Kõigil 16 haigel, kelle bronhides leidis trihhomoonaseid, tekkis positiivne nahareaktsioon vähemalt ühega kasutatud allergeenidest, seavastu 14 inimesel, kellel trihhomoonaseid ei sedastatud, oli intradermaaltset kõigi kasutatud allergeenidega negatiivne. Saadud tulemuste põhjal võib öelda, et inimese hingamisteedest isoleeritud trihhomoonased põhjustavad nii sensibiliseeritud katseloomadel kui ka nimetatud algloomadega nakatunud inimestel spetsiifilise allergia, mida on võimalik sedastada korpuskulaarsete allergeenide nahasisese manustamisega. Usaldusväärsete andmete saamiseks tuleb intradermaaltset tingimata sooritada samaaegselt mitmest trihhomoonaste tüvest valmistatud allergeenidega.

АЛЛЕРГЕННЫЕ СВОЙСТВА ТРИХОМОНАД, ОБИТАЮЩИХ В ДЫХАТЕЛЬНЫХ ПУТЯХ ЧЕЛОВЕКА

Для изучения аллергенных свойств трихомонад, обитающих в дыхательных путях человека, в первую очередь провели серию опытов на морских свинках, сенсибилизированных внутрибрюшинным, подкожным или внутрикожным введением аксенических культур трихомонад, выделенных как из дыхательных путей, так и из ротовой полости человека. Результаты проверяли путем внутрикожного введения корпускулярных аллергенов, приготовленных из тех же культур. Как показали полученные результаты, аллергенными свойствами обладали все штаммы трихомонад, — выделенные как из дыхательных путей, так и из ротовой полости, — причем возникшая аллергия была выражена сильнее к тому штамму, который использовали для сенсибилизации животного. Следует, однако, отметить, что при сенсибилизации морских свинок штаммами трихомонад, выделенных из дыхательных путей, внутрикожный тест аллергенами серотипных штаммов как *T. vaginalis*, так и *T. P. hominis* у всех морских свинок был отрицательным.

После опытов на животных, подтверждающих наличие аллергенных свойств штаммов *T. tenax*, был проведен внутрикожный тест корпускулярными аллергенами, приготовленными из аксенических культур пяти штаммов *T. tenax* (два изолированы из бронхов, один — из мокроты и два — из ротовой полости), у 30 больных с различными заболеваниями дыхательных путей.

Все больные были обследованы на наличие трихомонад как в бронхах, в мокроте и ротовой полости, так и в кишечном и урогенитальном трактах. Трихомонады в дыхательных путях были найдены у 16 обследованных, причем у них положительные результаты были получены по крайней мере с одним аллергеном, в то время как у 14 больных, у которых трихомонады не были обнаружены, результаты были отрицательными. Результаты внутрикожного теста у больных с легочной патологией, у которых трихомонады обнаружены в дыхательных путях, в большой мере зависели от использованного аллергена. Поэтому для получения достоверных данных при использовании внутрикожных проб необходимо вводить одновременно несколько аллергенов.

На основе полученных данных можно сказать, что трихомонады, обитающие в дыхательных путях человека, обладают аллергенными свойствами, вызывая в организме как подопытных животных, сенсибилизированных этими простейшими, так и людей, у которых трихомонады обитают в бронхах, специфическую аллергию, выявляемую путем внутрикожного введения корпускулярных аллергенов.