

From the Allergologic Department of the Faculty Policlinic in Prague, the Ophthalmologic Department, and from the Institute of Epidemiology and Microbiology in Prague, Czechoslovakia.

HYPERSENSITIVITY TO STREPTOCOCCI IN PATIENTS WITH RECURRENT UVEITIS

By

M. KOLEČKÁŘOVÁ, L. KLENKA & I. HÁNA

INTRODUCTION

Recurrent and chronic iridocyclitis is a comparatively common disease. In Europe, patients suffering from this affection occupy every year 5 per cent of all the beds in ophthalmological clinics, not to mention special institutions for climatic treatment. Many are admitted several times a year for hospital treatment which often lasts several weeks; the complaint keeps them away from their work repeatedly and for long periods of time, and may eventually lead to invalidism at an early age or even to complete blindness. Actually, 10-15 per cent of the inmates of Czechoslovak institutions for the blind had lost their eyesight in consequence of this disease.

There is a similarity between iridocyclitis in its recurrent form and certain allergic diseases. The attacks of the disease occur with significantly greater frequency in the spring and in the autumn, *i.e.* in the season of colds, generally lowered resistance of the organism and a higher incidence of streptococcal infections. Among the patients there are many who have been afflicted with different manifestations of allergy or with diseases of the joints, particularly rheumatic inflammatory involvement. On these facts the American ophthalmologist A. C. Woods, fifteen years ago, based his opinion that there is a causal nexus between certain forms of recurrent uveitis, recurrent iritis in particular, and hypersensitivity of uveal tissue to

different types of beta hemolytic streptococci (2-4). Other forms, particularly affections of the retina and the choroid have, in his opinion, a different etiology (in the first place toxoplasmosis and tuberculosis), although the possibility of sensitization of the tissues and an allergic response cannot be excluded even here. Offret, in his paper published several years later, claimed that a positive focal reaction on intradermal injection of streptococcal vaccine is evidence of allergic etiology in affections of the posterior segment of the uveal tract (1). To elucidate the role of streptococci, or rather hypersensitivity to streptococci, in the pathogenesis of uveitis, we devised a new modification of the method of assessing the degree of hypersensitivity. We attempted to express the degree of hypersensitivity numerically, and we compared the degree of hypersensitivity to streptococci of different groups and types in different groups of the population under parallel bacteriological and serological control. During this research certain hitherto unknown relationships were found which are reported in this paper.

MATERIAL AND METHODS

1. *Selection of Streptococcal Strains.*

As is well known, streptococci are divided according to their polysaccharide C-antigen into groups designated by the capital letters A-S. By far the most important in human pathology is group A—*Streptococcus pyogenes*, which is divided according to the protein M-antigen into types designated by the numbers 1-51. Since the type-specific M-antigen is by far the most important of all the known factors involved in the pathogenicity of streptococci it is necessary, in testing, to use the greatest possible number of streptococcal types. Testing with a mixed vaccine is inadequate, as the quantity applied in the test might not contain in threshold concentration the serological type to which the patient is sensitive, and thus an adequate reaction would not be obtained. On the contrary, with a higher concentration of the test vaccine, and particularly if the patient were hypersensitive to several types, a severe local reaction (necrosis) might ensue, possibly accompanied by a dangerous focal and even general reaction. Such an unphysiological and even harmful procedure must be avoided as it might cause serious trouble to the patient, and also give no information as to which of the antigens contained in the testing suspension is responsible for the reaction.

The streptococcal strains selected for testing were those types which, in this

country, were found most frequently. The follow up of their incidence has been carried on by the Streptococcus Reference Laboratory of the Institute of Epidemiology and Microbiology in Prague for a number of years and the incidence of these types in patients and carriers has been registered twice every year. Thus we were able to assemble in our testing set representatives of practically all the types occurring in this country. We believe that hardly anybody could be hypersensitive to a type not found in this country during the last years and for this reason we omitted these strains from our testing set.

The streptococci of the groups B-G only play an insignificant part in human pathology but, for purposes of control, hypersensitivity to one representative of each group was also investigated. A list of the groups and types of streptococci used is given in Table 1.

Most of these strains belong to *Streptococcus pyogenes* (group A—the most important streptococci) and for the sake of completeness a number of strains were included from the other groups which only play a minor part in human pathology. Only two of the strains used showed no beta-hemolysis.

TABLE 1
Streptococcal Strains Used.

Group	Type	Group	Type
A	1	A	18
	2		19
	3		22
	3, 13, B ₃₂₆₄		8, 25, Imp ₁₉
	4, 24, 26, 28, 29, 46		28 R
	5		30
	11		non-typable
	12	B	
	5, 11, 12, 27, 44	C	
	6	D	
9		G (minute)	
14		Str. salivarius	

2. Preparation of Bacterial Suspensions for Testing.

The lyophilized strain was inoculated on blood agar and its purity was assessed. From the plate, a pre-culture (5 ml of Todd-Hewitt broth) was inoculated and after 8 hrs. this pre-culture was used to inoculate 400 ml Todd-Hewitt broth. After 18 hrs. growth at 37° C, the culture was killed by heating (at 56° C for a period of 30 mins., in group D for 90 mins.) and washed twice with saline. Finally the bacterial suspension was adjusted to a constant density

of T 50 (50 per cent transmission) as measured with the Coleman spectrophotometer at wave length $520\text{ m}\mu$. This density roughly corresponds to 6 milliard microbes/ml. 0.25 per cent phenol was added as antiseptic. This test suspension was then diluted in the ratios of 1:10, 1:100, 1:1000 and 1:10,000. The undiluted stock suspensions were kept at -20°C , the diluted vaccines (*i.e.* before use) at $+4^{\circ}\text{C}$.

3. *Technique of Testing and Reading of Reactions.*

The testing was performed intradermally on the volar side of the arm. The advantage of this method is that it avoids Head's zones in the skin of the back. The reactions of the tests were read (*i.e.* their diameter was measured in mm) after 15 mins. (urticarial wheal), 6 hrs., 24 hrs. and 48 hrs. (erythema, induration). Before testing and at the above mentioned intervals after testing, the focal ophthalmic reaction was also studied by the ophthalmologist. Only exceptionally is it possible to study so closely *in vivo* with the microscope the reaction of sensitised tissue after the injection of an antigen. A Zeiss slit-lamp was used to observe whether cellular elements appeared or increased in number in the aqueous (activating reaction) or whether they decreased in number or actually even temporarily disappeared (mitigating reaction). The activating reaction was observed in 71.7 per cent of uveitic patients, the mitigating reaction in 7.7 per cent. At the same time attention was paid to the possible occurrence of a general reaction.

The testing was performed at weekly intervals using, every time, a test suspension ten times more concentrated than had been applied in the previous sitting. This cautious procedure was resorted to for two reasons. Firstly, it is much safer for the patient to start with low doses which are gradually increased, so that necrosis of the skin, severe focal reactions (acute iridocyclitis) and general symptoms may be avoided. Nevertheless, the initial dose for 1 test ($T\ 50 \times 10^{-4}$, *i.e.* roughly 30,000 microbes in 0.05 ml) is adequate and in the group of 178 uveitic patients dealt with in this paper, the local reaction to one of the 24 tests at least was found to be positive in 56.1 per cent of the subjects examined. The second reason for testing with increasing concentrations is that the threshold dose causing a local skin reaction and a focal ophthalmic reaction (which must be determined for the correct dosage of the therapeutic vaccine) may be found more easily than by the method of decreasing concentrations used by *Woods* and later by *Offret*. Another reason for testing with four concentrations is the fact that the size of the local reaction does not always increase proportionally with the concentration of the test suspension. Occasionally (in the negative phase) the size of the local reaction, on application of more concentrated test suspension, is no greater than the size of the local reaction on application of less concentrated test suspension.

Fig. 1 shows four main possibilities of the increase in size of local reactions on application of the 4 concentrations of the test suspension.

Sizes of skin reactions on application of different concentrations of test suspension.
(For explanation see text).

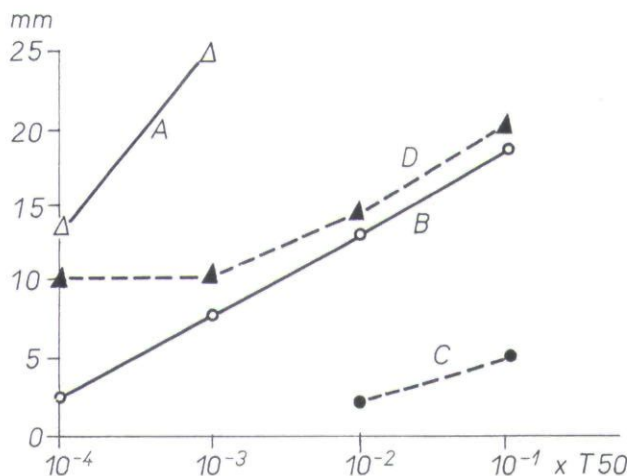


Fig. 1.

- A) High degree of hypersensitivity.
- B) Size of skin reaction increases in proportion with the increase in concentration of test suspension.
- C) Low degree of hypersensitivity.
- D) Skin reaction on application of test suspension of concentration $T 50 \times 10^{-4}$ was 10 mm in diameter. On application of test suspension of concentration $T 50 \times 10^{-3}$ the resulting skin reaction was equal in size and only increased on application of higher concentrations of the test suspension.

For these reasons 4 tenfold concentrations were used in testing and in order to obtain as complete as possible a picture of the degree of hypersensitivity of a given subject to a given serological type, all the 4 results were taken into account.

In the first week the testing was done with test suspensions of concentration $T 50 \times 10^{-4}$. In the following week, the concentration used was $T 50 \times 10^{-3}$ and the tests were injected into the skin of the other arm. Testing with types which in the previous sitting had had a reaction 15 mm in diameter or more, was discontinued. In the third week the concentration used was $T 50 \times 10^{-2}$ and in the fourth week it was $T 50 \times 10^{-1}$.

Considering that the tests were actually repeated four times with the same antigens and their results were correlated in all cases, that the same syringes were always used and the tests were always performed and read by the same worker (without seeing the results of the preceding tests), the tests may, from the point of view of statistics, be considered as standard within the range of errors which do not influence the final result.

RESULTS

1. *Numerical Evaluation of the Results.*

The degree of hypersensitivity was evaluated according to the size of the skin reaction and according to the concentration of the test suspension. The degree of hypersensitivity to a given type is directly proportional to the size of the skin reaction and inversely proportional to the concentration of the test suspension applied. The authors devised a numerical schema for the evaluation of the size of the skin reactions (see Table 2), and on the basis of the values obtained, they attempted a numerical expression of the degree of hypersensitivity in a given subject.

TABLE 2

Evaluation of the Size of the Skin Reaction on Application of Test Suspension of Different Concentrations.

Concentration of test suspension	Size of skin reaction in mm			
	7-8	9-10	11-14	15 and more
T 50×10^{-4}	4	5	6	7
T 50×10^{-3}	3	4	5	6
T 50×10^{-2}	2	3	4	5
T 50×10^{-1}	1	2	3	4

The sum of the points obtained by a given subject for a given streptococcal type in all the four concentrations, represents the degree of hypersensitivity to that type. The sum of the results for all the 24 types is a number which represents the degree of hypersensitivity of a given subject to the streptococci tested (selected strains). This number was designated as the streptococcal coefficient (SC). This system is superior to expressing the size of the reactions by a system of pluses as it allows a numerical evaluation of the degree of hypersensitivity. At the same time the system makes it possible to evaluate the reactions to all the four concentrations by a single number. Papules and minute necroses, which were only observed in 0.5 per cent of all the reactions and only in reactions over 15 mm in diameter, were not taken into account in the

evaluation. In this system of evaluation, the uveitic patients tested (178 in number) obtained the following number of points:

in the first sitting—a total of	2406 pts.	(7.85 per cent)
in the second sitting—a total of	6303 pts.	(20.57 per cent)
in the third sitting—a total of	9568 pts.	(31.23 per cent)
in the fourth sitting—a total of	12355 pts.	(40.33 per cent)

so that the average SC for one uveitic patient was 172.08 (30,632:178).

Examples of Evaluation.

1. Size of skin reaction in the 1st sitting = 5 mm = 0 pts.
(not evaluated)
Size of skin reaction in the 2nd sitting = 7 mm = 3 pts.
Size of skin reaction in the 3rd sitting = 12 mm = 4 pts.
Size of skin reaction in the 4th sitting = 15 mm = 4 pts.

Total = 11 pts.

2. Size of skin reaction in the 1st sitting = 10 mm = 5 pts.
Size of skin reaction in the 2nd sitting = 20 mm = 6 pts.

Testing with this type was discontinued for reasons mentioned in the chapter on technique of testing. For the 3rd and 4th sitting, it was evaluated as 6 pts. (*i.e.* the maximum number of points obtained in the last sitting). This type obtained a total of 5+6+6+6 pts. = 23 pts.

2. *The Time Necessary for the Local Reaction to Reach its Maximum (Maximum Local Reaction—MLR).*

Local reactions in all the 178 patients were of the delayed type. Only in one female subject (who is not included in this series) were the reactions of the early (immediate) type (urticarial wheal). In testing a practically homogeneous group of 178 uveitic patients with 24 types of streptococci in 4 different concentrations, and reading the local reactions at 4 different intervals (15 mins., 6 hrs., 24 hrs., and 48 hrs.), sufficient data were assembled to enable us to determine the time when the local reaction attains its maximum. Where the size of the local reaction was equal in two consecutive readings, it was taken for granted that its maximum (MLR) fell between the two observations. It is, of course, possible that the MLR

might have been attained earlier or later after the reading. It can, however, be proved statistically that with 15,718 observations these variations would be equally frequent.

The MLR was attained in testing with

T 50×10^{-4} in 102,371 hrs. (no. of tests applied: 4248)—average: 24.09 hrs.

T 50×10^{-3} in 122,920 hrs. (no. of tests applied: 4140)—average: 29.69 hrs.

T 50×10^{-2} in 125,921 hrs. (no. of tests applied: 3985)—average: 31.59 hrs.

T 50×10^{-1} in 113,196 hrs. (no. of tests applied: 3345)—average: 33.84 hrs.

This means that the time necessary for the skin reaction to reach its maximum increases with the concentration.

3. *The Relationship between the Degree of Hypersensitivity to Streptococci (Expressed in Terms of SC) and the Time Necessary for the Skin Reaction to Reach its Maximum (Expressed in Terms of MLR).*

After determining SC we tried to elucidate the relationship of this coefficient to certain serological reactions, to the age of the patient, to the number of attacks etc. The evaluation of these relationships will be reported elsewhere. (It is interesting to note the linear relationship between SC and MLR (see Table 3).

From Table 3 it may be seen that in patients with a higher degree of hypersensitivity to the streptococcal strains used, a longer period of time is necessary for the skin reaction to reach its maximum.

4. *Comparing the Degree of Hypersensitivity in Different Groups of the Population.*

Three different groups of the population were examined by the abovementioned method of intradermal testing to determine the degree of hypersensitivity to different groups and types of streptococci (beta-haemolytic streptococci in particular).

1. A group of male adolescents, all of them 15 years of age and living in Prague. All had a "normal" history of strep-

tococcal infection, similar to that of all adolescents of that age living in Central Europe. 5 had had scarlet fever, 4—otitis and 11 had had tonsillitis in the last few months.

TABLE 3

The Relationship between the Degree of Hypersensitivity to Streptococci (Expressed in Terms of SC) and the Time Necessary for the Local Skin Reaction to Reach its Maximum (Expressed in Terms of MLR).

MLR in hrs.	SC in points														Σ
	0	50	100	150	200	250	300	350	400	450	500	550	600		
15	6	2												8	
	2	10	1											13	
20	9	11	12	1			1	1						35	
25		13	7	8	8	1			1					38	
		3	11	12	7	6	5							44	
30		2	2	2	7	7	1	4		2				27	
40			1			2	3	1	2					9	
									1	1	1	1		4	
45															
Σ	17	41	34	23	22	16	10	6	4	3	1	1		178	

2. A group of 14 male adolescents aged 16–19. During the last 10 years 12 of them had had one attack of rheumatic fever, one had had two attacks and one had had as many as three. At least two years had elapsed in all cases since the last attack. All were under medical control and under long-term treatment with repository penicillin. Besides the manifest sequelae of infection with beta-hemolytic streptococci, all had a history of streptococcal infection similar to that of the subjects in the first group. 13 had had frequent tonsillitis up to the time of tonsillectomy, 2 had had scarlet fever and 6 had had otitis.

Both these groups served as controls.

3. The third group was formed of 178 patients suffering

from different forms of recurrent and chronic inflammations of the uveal tract. Their ages ranged from 14 to 72 years, the average being 47 years. Both sexes were represented nearly equally. The different forms of the disease were recurrent iritis, episcleritis, tenonitis, sclerokeratitis, cyclitis and recurrent inflammations of the posterior segment of the uvea. The disease, of course, changes its localisation from one attack to another; the clinical picture changes. All the patients had been admitted many times for hospital treatment, many had been treated in institutions for climatic treatment and had undergone treatment with antibiotics, corticosteroids, tuberculostatics, antimalarials, Daraprim etc. As the clinical picture of uveitis with the presumptive etiology of hypersensitivity to streptococci is not clearly defined (and it is an open question whether it is at all uniform), all the subjects were examined by the same method, the degree of hypersensitivity to streptococci was determined and compared with that of the other groups. The results may be seen in Table 4.

TABLE 4

The Degree of Hypersensitivity to Streptococci (Expressed in Terms of SC) in the two Control Groups (Normal Population and Rheumatic Patients) Compared with that of Uveitic Patients.

	n	SC	Average	95% confidence limits
Normal	17	0-200	60.65	32.00-89.18
Rheumatic .	14	21-203	83.42	53.22-113.50
Uveitic	178	6-588	171.01	153.97-188.05

DISCUSSION

The average degree of hypersensitivity to different strains of beta-hemolytic streptococci was found to be higher in patients with chronic and recurrent iridocyclitis than in representatives of the normal population and even in subjects who had had rheumatic fever (and where, according to the present state of knowledge, hypersensitivity to streptococci may be assumed). During testing very frequent focal reactions were

observed. From these two facts, the authors conclude that streptococci play a part in the pathogenesis of endogenous uveitis more frequently than has hitherto been presumed and that in cases where the tests are positive, it might be worth while—regardless of the presumptive “clinical etiological diagnosis”—to attempt a course of treatment by specific desensitisation. It is understood that, in this therapy, those of the strains found in the foci of the patient to which the patient was found to be hypersensitive, would also have to be taken into account.

SUMMARY

The authors discuss the importance of the problems of recurrent and chronic inflammations of the uveal tract. Most of them may be presumed to be due to hypersensitivity. It appears that different types of beta-hemolytic streptococci play an important part in the pathogenesis of this disease. The authors worked out an original schema for determining the degree of hypersensitivity to streptococci by intradermal tests. On the basis of the evaluation of every skin reaction on application of the test suspensions in four different concentrations, with simultaneous control of the focal ophthalmic reactions, they expressed the degree of hypersensitivity of a given subject by a number which they designated the streptococcal coefficient (SC). They pointed out the relationship between the time necessary for the skin reaction to reach its maximum and the concentration of the test suspension, and the relationship between this period of time and the total degree of hypersensitivity of the patient. They compared the degree of hypersensitivity to streptococci in the normal population, in rheumatic patients and in a large group of patients with chronic and recurrent forms of uveitis, and on the basis of mathematical evaluation they demonstrated that the large majority of uveitic patients show a high degree of hypersensitivity to different types of beta-hemolytic streptococci.

REFERENCES

1. *Offret, G. et al.*: L'uvéite streptococcique existe-t-elle? Arch. d'Ophtal. 22, 565-590, 1962.
2. *Woods, A. C.*: Use of specific streptococcus vaccine in nongranulomatous uveitis. Arch. Ophthal. 50, 129-146, 1953.
3. *Woods, A. C.*: A further study of streptococcus vaccines in nongranulomatous uveitis. Arch. Ophthal. 56, 749-755, 1956.
4. *Woods, A. C.*: Endogenous uveitis. The Williams & Wilkins Co., Baltimore, 1956.

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.