LYMPHOCYTE-TRANSFORMING ACTIVITY OF STREPTOCOCCI BELONGING TO VARIOUS LANCEFIELD GROUPS

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THE lymphocyte-transforming activity of preparations of streptolysin S was first reported by Hirschhorn *et al.* (1964), and it has since been demonstrated that the mitogen is not the haemolysin itself (Plate and Amos, 1969; Taranta, Cuppari and Quagliata, 1969; Taylor, 1969). The production of the mitogen by streptococci does not depend on the presence of RNA used to induce formation of streptolysin S (Plate and Amos), but detailed studies of the conditions of culture under which the active material is formed have not been published. There is no information on the release of mitogen by streptococci other than the group-A strains (no. C203S and its non-haemolytic mutant no. C203U) used by Bernheimer (1949) in his original studies on streptolysin S.

Current interest in the mitogen is not only with respect to its action on lymphocytes *in vitro* but also to its possible effect on the immune response in streptococcal infections (Taranta *et al.*). In this study, 27 strains of group-A streptococci and 39 strains of other Lancefield groups have been examined for mitogenic activity.

MATERIALS AND METHODS

Streptococci. Strain no. C203S was originally obtained from Professor A. W. Bernheimer. All other strains were provided as freeze-dried cultures by Dr W. R. Maxted of the Streptococcus Reference Laboratory, Colindale, London; these were reconstituted with Todd-Hewitt broth, incubated at 37°C overnight, and tested for purity by culture on blood agar.

Culture of streptococci. Todd-Hewitt broth, 10 ml in 15-ml screw-capped bottles, was inoculated with 0.5 ml of a fresh broth culture of streptococci and incubated overnight at 37°C. The turbidity of the cultures was determined in a spectrophotometer (650 nm) and only those with an optical density of 0.8–1.0 were used. These cultures were centrifuged and the supernatant fluids were removed and sterilised by filtration through Oxoid membranes (0.45 µm) in Hemming's centrifugal filters.

Investigation of mitogenic activity. Human peripheral-blood lymphocytes were obtained and cultured as previously described (Taylor). From the culture supernatants to be tested 0.1 ml was added to each of two lymphocyte cultures containing 10⁶ cells in 1 ml of medium. For each test, positive and negative control cultures were also examined; the negative control received uninoculated broth and the positive control the supernatant from a broth culture of strain Blackmore, known to be strongly mitogenic. After incubation for 24 hr, 0.1 µCi of thymidine-2-C¹⁴ (Radiochemical Centre, Amersham, Bucks.) was added and incubation was continued for another 24 hr. The number of surviving cells in each culture was then determined in a haemocytometer; 0.05-ml volumes were added to equal volumes of leucocyte-diluting fluid. The cultures were filtered through Swinnex filters with glass-fibre filter disks (Millipore Ltd) as described by Sorensen, Andersen and Giese (1969). The disks were transferred to scintillation-counting vials with 10 ml phosphor {0.5 per cent. butyl-PBD (Ciba

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Ltd) in toluene}. Counts were corrected for quenching by external standardisation, and the activity of each culture was expressed as disintegrations per minute (d.p.m.) per 10^6 surviving lymphocytes.

The thymidine uptake induced by 0.1 ml of test supernatant was compared with the thymidine uptake in the same batch of lymphocytes induced by 0.1 ml of positive-control broth supernatant, to give a "mitogenic index":

$$\text{mitogenic index} = \frac{T-N}{P-N} \times 100$$

where T = mean activity (d.p.m.) of two test cultures,

P = mean activity (d.p.m.) of two positive control cultures,

N = mean activity (d.p.m.) of two negative control cultures.

In most cases a third lymphocyte-culture tube for each supernatant was included to allow microscopic examination for transformed cells after 5 days' incubation. The volume of supernatant added was 0.1 ml and the technique was as described previously (Taylor).

RESULTS

Culture supernatants from all 27 strains of group-A streptococci gave a high mitogenic index (table I). To exclude the possibility that the increased thymidine uptake was due to transformation of lymphocytes of sensitised donors by

TABLE I
*Mitogenic activity on human lymphocytes of supernatants from
broth cultures of group-A streptococci*

Strain number	M-type	Mitogenic index	Percentage of transformed cells
8198*	1	312	...†
8322*	2	210	...
100064	3	72	...
R70/3067	3	78	70
R70/3251	3	38	50
100065	5	146	...
8302*	6	116	...
100067	9	189	...
R59/2653	11	260	...
100068	11	47	...
R70/2179	11	28	60
R70/1555	11	86	70
100085	12	114	...
100070	15	54	...
8320*	18	80	...
R68/1663	22	48	70
R71/80	22	74	70
R68/616	22	82	70
R68/98	22	83	70
8301*	23	57	...
R70/740	24	157	70
R63/10	24	75	70
R68/3306	26	60	70
R68/2568	26	65	70
8195*	30	102	...
8227*	36	82	...
8230*	46	73	...

* = NCTC number.

† ... = Cells not examined microscopically.

TABLE II
Mitogenic activity of supernates from broth cultures of streptococci
of various Lancefield groups

Group	Serotype or species	Strain designation	Mitogenic index	Percentage of transformed cells	Mitogenic activity*
B	Ia	O90	3.9	15	—
B	Ib	H36B (RCL)	1.6	3	—
B	II	18RS21 (RCL)	4.6	15	—
B	III	D136C	3.1	2	—
C	<i>Str. zooepidemicus</i>	D181	8.7	3	—
C	<i>Str. zooepidemicus</i>	R70/2246	117	50	+
C	<i>Str. equisimilis</i>	NCTC4540	90	60	+
C	<i>Str. equi</i>	R70/2244	300	65	+
C	<i>Str. equi</i>	53/54	108	67	+
C	<i>Str. equi</i>	35/55	62	45	+
C	<i>Str. equi</i>	37/55	100	70	+
D	<i>Str. faecalis</i>	775	1.8	2	—
D	<i>Str. faecium</i>	HGH511	2.9	3	—
D	<i>Str. durans</i>	98D	0.8	5	—
D	<i>Str. bovis</i>	Rosalie	5.3	8	—
E		49116	1	2	—
F		R70/2178	1.8	1	—
F		O'Mahoney	1	6	—
G		R70/2496	48	40	+
G		R70/2433	35	75	+
G		Valente	6	8	—
H		Blackburn	7.2	3	—
H		3437/48	14	2	—
H		Challis	1	2	—
H		5042/48	1	1	—
H		868/49	5.4	4	—
K		Levy	1.5	5	—
K		Turner	6.8	2	—
K		R51/551	1	1	—
L		SHC16	6.6	1	—
L		SHC146	7.8	3	—
L		R65/3365	4.2	4	—
L		R66/2047	6.7	8	—
L		SHC282	5.4	5	—
L		NCTC7868	1	1	—
N	<i>Str. lactis</i>	NCTC6681	1	7	—
P		54/851	1	6	—
Q		E6844	8.9	3	—
R		735	9.1	8	—

* + = Mitogenic activity indicated by mitogenic index >25 or by >20 per cent. of transformed cells.

— = No mitogenic activity.

specific antigen, the mitogenic index was determined for lymphocyte cultures from Mantoux-positive donors, in the presence of PPD*. The optimum concentration of PPD for maximal thymidine uptake during the last 24 hr of a 72-hr culture period was 125–250 μ g. With PPD concentrations within this range, the mitogenic index was never greater than 20 and the number of transformed cells seen in smears was not more than 15 per cent. Lymphocytes from two donors were incubated with streptolysin O and showed much lower levels of stimulation. It was concluded that a mitogenic index of more than 25, or the presence of more than 20 per cent. lymphoblasts gave a strong indication of non-specific transformation due to the presence of streptococcal mitogen. All group-A strains investigated were therefore considered to be producers of streptococcal mitogen in broth culture.

The strains of other Lancefield groups examined in this study, with the exception of some group-C and group-G strains, showed low mitogenic indices (table II) and it seems that these strains do not produce the mitogen in significant amounts. Six of these seven group-C strains and two of the three group-G strains examined produced mitogenic activity.

DISCUSSION

The results of these tests with human lymphocytes indicate that the production of the streptococcal non-specific lymphocyte mitogen may be confined to strains belonging to the Lancefield groups A, C, and G. The role of the mitogen in human infection is not known, but it is noteworthy that the common pathogenic streptococci of man belong to these three groups. Study of the mitogenic action of culture supernatants from strains of other Lancefield groups on lymphocytes of their typical hosts might be rewarding.

Some speculation can be made on the significance of the mitogen *in vivo*. For example, Ginsburg *et al.* (1968) produced lesions in the myocardium of rabbits by the intratonsillar injection of a pool of extracellular products from a group-A streptococcus. This pool contained deoxyribonuclease, ribonuclease, nicotinamide-adenine-nucleotidase, hyaluronidase, and streptokinase. The mechanism by which the lesions were induced was not understood; but in the light of the present study it seems likely that the pool used by Ginsburg *et al.* was mitogenic as the preparation was derived directly from unpurified culture supernatants. The possibility that the mitogen is involved in the tissue-damaging process should therefore be considered.

Activated lymphocytes are known to release several soluble factors, termed "lymphokines" by Dumonde *et al.* (1969). These include a lymphocyte mitogenic factor, an inflammatory factor, and a cytotoxic factor (Kolb and Granger, 1968). These lymphokines are possibly involved in the inflammation of delayed hypersensitivity and in the rejection of tissue allografts.

If the streptococcal mitogen activates lymphocytes *in vivo* as well as *in vitro*, these potentially cytotoxic cells may migrate into the myocardial tissue and there

* Tuberculin, purified protein derivative (PPD), 50,000 IU per mg (Ministry of Agriculture, Central Veterinary Laboratory, Weybridge, Surrey).

initiate the type of lesion reported by Ginsburg *et al.* The possibility that a similar reaction occurs after streptococcal infection of the human pharynx should be considered in relation to the aetiology of rheumatic fever. A study of the effects of activated lymphocytes *in vivo* has therefore been started.

SUMMARY

Broth cultures of 27 strains of group-A streptococci and 39 strains of various other Lancefield groups were tested for lymphocyte-transforming activity. All the group-A strains and most of the group-C and group-G strains were strongly mitogenic. The possible significance of the mitogen in streptococcal infection is discussed.

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