Cytotoxicity of Antiserum Sensitized to Hemolytic Streptococcal M Protein Fraction on Cultured Myocardial Cells

MASAO YOSHINAGA, M.D., SYUJI TAKEI, M.D., MASASHI HOKONOHARA, M.D.
KOICHIRO MIYATA, M.D., AND TAMOTSU TERAWAKI, M.D.

We have studied cytotoxicity of antisera of monkeys sensitized to streptococcal M protein fraction, which was prepared by the method of Lancefield et al., on cultured myocardial cells. These antisera exerted a significantly higher cytotoxic effect on the myocardial cells than normal monkey sera, and this cytotoxic effect seemed to be organ-specific. In the presence of normal monkey lymphocytes, the antisera had a cytotoxic effect on myocardial cells, but normal sera in the presence of normal monkey lymphocytes had no cytotoxic effects.

These data suggest that M protein fraction of hemolytic streptococcus plays an important role in the pathogenesis of rheumatic carditis.

IT is a well-known fact that hemolytic streptococci are connected with the development of rheumatic fever. However, the etiology of the disease is unknown.

On the basis of the finding of Kaplan et al.¹ that there is a cross reaction between hemolytic streptococci and heart tissue, Terawaki et al.²⁻⁵ used the hemolytic streptococcal M protein fraction to produce what may be called rheumatic carditis in the "Macaca iris" monkeys.

In order to investigate further the mechanism of cytotoxicity in these experimental series, we studied cytotoxicity of the anti-M protein fraction antibody on cultured myocardial cells.

METHODS

Antigen: M protein fraction of Group A, type 12, hemolytic streptococci was prepared

according to the procedure of Lancefield et al.⁶

Antisera: Sensitization procedure for the monkeys was carried out as follows: the first, 5 mg of M protein fraction, suspended with 5 ml of PBS and equal volume of incomplete Freund's adjuvant (IFA), was administered subcutaneously. Two to 3 weeks after the injection, 1 mg of M protein fraction in PBS (without IFA) was administered intravenously at 2 to 3 weeks intervals. Antibodies were confirmed by immunodiffusion with M protein fraction. Untreated monkey sera were used as the control. Sera were all inactivated by heating at 56°C for 30 minutes, and diluted with growth medium.

Complement: Guinea pig whole complement (Cordis Laboratories, Miami) was used.

Lymphocytes: After collecting heparinized peripheral blood from the monkey, lymphocytes were separated by the Ficoll-Conray density gradient centrifugation technique and adjusted to 1×10^6 lymphocytes/ml.

Myocardial cells: Cultured myocardial cells were prepared according to Kasten's method⁷ with a necessary modification. That is, the ventricles of newborn ddY mice were taken out

Key Words:

Streptococcal M protein fraction
Cultured myocardial cells
Cytotoxicity test
Complement-dependent cytotoxicity
ADCC

Department of Pediatrics, Faculty of Medicine, Kagoshima University, 1208-1 Usuki-cho, Kagoshima 890, Japan

Fig.1. Cytotoxicity of anti-M protein serum on cultured myocardial cells.

Fig.2. Cytotoxicity of anti-M protein serum: effect of preincubation with cultured cells.

aseptically within 3 days after birth. After dispersing the ventricles in Hanks CMF solution containing 0.1% trypsin, supernatants were washed 3 times and suspended in MEM (Grand Island Biological Co., New York) containing 10% FCS. A final concentration of 2.5×10^4 cells in 0.2 ml were seeded onto each well of a Falcon 3040 (Falcon Plastic Division of BioQuest, Oxnard).

Fig.3. Cytotoxicity of anti-M protein serum: effect of normal lymphocytes.

Cytotoxicity test: After 18 to 24 hours of cultivation of myocardial cells, $2 \mu\text{Ci/well}$ of ^{51}Cr was added to them, and they were cultured overnight. After the labelled cells were washed 3 times with growth medium, sera or lymphocytes were added. The reaction time was set at 3 hours for the cytotoxicity test of sera and 24 hours for that of lymphocytes. The degree of cytotoxic effect was expressed by means of a release index as follows:

$$\% \text{ Release} = (\text{test release cpm} - 0 \text{ hour release cpm}) / (\text{total incorporation cpm} - 0 \text{ hour release cpm})$$

$$\text{Release index} = (\text{test \% release} - \text{spontaneous \% release}) / (\text{maximum \% release} - \text{spontaneous \% release})$$

RESULTS

Cytotoxicity of Anti-M Protein Fraction Sera

The release index of antisera with complement was 30.1 ± 8.7 , significantly higher than that of normal sera with complement, 1.3 ± 9.2 . Antisera without complement exerted no cytotoxic effects. When this antisera were diluted, the degree of cytotoxicity fell off with dilution, $\times 5$, $\times 10$, and $\times 20$ (Fig. 1).

In order to investigate the organ specificity,

TABLE I CYTOTOXICITY OF SENSITIZED SERUM AND LYMPHOCYTES ON CULTURED MYOCARDIAL CELLS

	Cultured cell	Antigen	Relation-ship*	Cytotoxicity		
				C'-dependent	lymphocyte-mediated	ADCC
Thompson, et al. ⁸ (1971)	rabbit	rabbit heart homoge.	hetero	+		
		rabbit heart homoge.	allo	—		
	rat	rat heart homoge.	hetero	+		
		rat heart homoge.	allo	—		
Friedman, et al. ⁹ (1971)	rat	rabbit heart homoge.	allo		+	
		rat heart homoge.	allo		+	
Kyogoku, et al. ¹⁰ (1973)	rat	mouse heart homoge.	allo		—	+
		streptoc.	allo	+		
		streptoc.	hetero	+		
Iida, et al. ¹¹ (1976)	human		allo		+	
Friedman, et al. ¹² (1976)	rat	rat heart homoge.	hetero	+		
Yang, et al. ¹³ (1977)	guinea pig	streptoc. homoge.	allo	—	+	—
		streptoc. M-Protein (type 12)		—	—	—
Ghose, et al. ¹⁴ (1977)	human		auto	—	—	
	mouse		hetero	—		
Weppe, et al. ¹⁵ (1977)	rat	streptoc. Zellysate (type 1, 19)	allo		+	
		streptoc. M-Protein (type 1, 19)	allo		—	

*: relationship between cultured cells and antiserum

this antisera were preincubated with both cultured myocardial cells and cultured skin cells from ddY newborn mice before cytotoxicity tests. The release index of antisera after preincubation with myocardial cells decreased to 14.9 ± 3.5 in one hour and to 16.5 ± 0.7 in 3 hours. However, the release index after preincubation with skin cells was 30.7 ± 2.0 in 3 hours, which showed little difference from the value before preincubation (Fig. 2).

Effect of Normal Lymphocytes: Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

The cytotoxicity of antisera or normal sera in the presence of normal lymphocytes were examined. In the presence of normal lymphocytes, the release index of antisera was 20.6 ± 2.3 , which showed significantly high cytotoxic effect compared with that of normal sera, -3.5 ± 2.8 . Normal lymphocytes alone had no cytotoxic effect on cultured myocardial cells (Fig. 3).

DISCUSSION

It is generally known that anti-hemolytic streptococcal antibodies or lymphocytes having a cross-reaction with organ antigen are connected with poststreptococcal rheumatic carditis.

However, no definite results have been obtained yet as to what cell fraction of hemolytic streptococcus is involved.

Many experiments have been carried out to determine whether antistreptococcal antibodies or antimyocardial antibodies exert cytotoxic effects on cultured myocardial cells, as shown in Table I.

We studied cytotoxicity of antisera against the M protein fraction prepared by the Lancefield's method, and demonstrated that these antisera had both complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC). These results are understandable since there is cross antigenicity between the M

protein fraction and the heart muscle.

As regard the organ specificity, the anti-M protein fraction antibody absorbed by myocardial cells clearly decreased in cytotoxicity, which suggests that this antiserum also has organ specificity.

As is presumed from Table I, the more heterogenic the experimental series is, the stronger cytotoxicity tends to be presumed. We will be making a study using the allogenic or autologous series in the future.

REFERENCES

1. KAPLAN MH, MEYESERIAN M: An immunological cross reaction between group A streptococcal cells and human heart tissue. *Lancet* i: 706, 1962
2. TERAWAKI T: Infections and body responses: Study on rheumatic fever. *J Jpn Med Assoc* 83: 21, 1980 (in Japanese)
3. NAGATA Y: Experimental study on rheumatic carditis induced by strain 5 and 12 hemolytic streptococci. *Igaku Kenkyu* 45: 32, 1975 (in Japanese)
4. HOKONOHARA M: Experimental study with strain 12 hemolytic streptococcus and its cell components on rheumatic carditis. *Ryumachi* 14: 192, 1974 (in Japanese)
5. MIYATA K: Experimental studies on rheumatic fever: Relationship between streptococcus hemolytics and body response. *Igaku Kenkyu* 43: 29, 1973 (in Japanese)
6. LANCEFIELD RC, PERLMANN GE: Preparation and properties of type specific antigen isolated from group A type 1 hemolytic streptococcus. *J Exp Med* 96: 71, 1952
7. KASTEN FH: Tissue Culture. Academic Press, New York, 1973, p 72
8. THOMPSON A, HALBERT SP: The cardiac autoimmune system. III. Studies on the cytotoxicity of heart antibodies for pulsating rabbit and rat heart cells in tissue culture. *Int Arch Allergy* 40: 274, 1971
9. FRIEDMAN I, LAUFER A, RON N, DABIES AM: Experimental myocarditis: In vitro and in vivo studies of lymphocytes sensitized to heart extracts and group A streptococci. *Immunology* 20: 225, 1971
10. KYOGOKU M, WATANABE M, NOSE M: Regulatory factors on immunocytolysis of cardiac cells and mesenchymal cells. *Jpn Circul J* 39: 433, 1975
11. IIDA N: Cytotoxicity assay against cultured heart cells in rheumatic heart disease. *Jpn Circul J* 42: 886, 1978
12. FRIEDMAN I, LAUFER A: Electron microscopical studies of the effect of antiheart antibodies and complement on beating heart cells in culture. *J Moll Cell Cardiol* 8: 641, 1976
13. YANG LC, SOPREY PR, WITTNER MK, FOX EN: Streptococcal-induced cell-mediated immune destruction of cardiac myofibers in vitro. *J Exp Med* 146: 344, 1977
14. GHOSE T, MAMMEN M: Interaction in vitro between myocardial cells and autologous lymphocytes and sera from patients with rheumatic carditis. *Chest* 71: 730, 1977
15. WEPPE C-M, WAGNER M, KÜHEMUND O, SCHMITZ HH, KÖHLER W: Das Vorkommen kreuzreaktiver Antigene in Herzmuskelgewebe und Streptococcus Pyogenes. IV. Zytotoxischer Effekt von Lymphozyten auf Primärkulturen von Herzmuskelzellen nach In-vivo-Sensibilisierung mit Streptokokken der Gruppe A. *Z Immun-Forsch* 153: 120, 1977