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Effect of M Protein of *Streptococcus pyogenes* on Macrophage Functions*)

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ABSTRACT

Streptococcal M protein was studied to ascertain its effect on macrophage functions such as oxidative metabolism, phagocytosis, and microbicidal activity. No significant stimulating activity of M protein against macrophage functions such as H_2O_2 or O_2^- releasing ability in response to phorbol myristate acetate triggering was noted. M protein did not enhance the macrophage-mediated host risistance to infection with *Listeria monocytogenes*. However, macrophages elicited with M protein showed a somewhat more enhanced phagocytic activity than did the resident macrophages.

M protein which is closely associated with virulence of group A streptococci is known to modulate human lymphocyte functions such as the mitogenic activity against T and B lymphocytes[15,16], and the natural killer cell activity[18]. M protein acts as an antiphagocytic factor against granulocytes as well as macrophages, presumably because of its toxic effect against these cells[1,4]. The detailed mechanisms with regard to the antiphagocytic and cytotoxic action of M protein are not fully elucidated. Reports that M protein-positive strains of streptococci specifically adhere to epithelial surfaces[2] and that M protein combines stoichiometrically with fibrinogen in a specific manner[8], suggest the existence of receptor sites for M protein on the membrane surface of certain cells. From these findings, there is a possibility that M protein alters macrophage cell functions by its specific binding to the receptor sites. In relation to this, we previously reported that certain lectins such as wheat germ agglutinin and concanavalin A showed a triggering or suppressive action on macrophage H_2O_2 releasing function through their binding to the specific receptor sites[14,17]. In the present study, we examined the effect of M protein of *Streptococcus pyogenes* strain Su on macrophage cell functions such as release of active oxygens, microbicidal activity, and phagocytosis.

M protein purified by the method of Lancefield[9] was donated for this study by Chugai Pharmaceutical Co., Tokyo. Macrophage monolayer culture from peritoneal exudate cells elicited with either M protein (20 μg/mouse), OK-432[13] (0.1 mg/mouse) obtained from Chugai Pharmaceutical Co., or ovalbumin (20 μg/mouse) was prepared on plastic culture dishes (35 or 50 mm), as described in a previous paper[17]. Macrophage phagocytosis of latex particles was performed in Eagle's minimal essential medium supplemented with 0.4% glucose, 1 mM pyruvate, 0.01 volume of non-essential amino acid (Difco Laboratories, Detroit, Michigan, U. S. A.) and 10% fetal bovine serum (MAB, Walkersville, MD, U. S. A.), in the presence of 100 μg/ml of latex particles (Sigma Chemical Co., St. Louis, Mo, U. S. A.) at 37°C for 15 min in a CO_2 incubator (5% CO_2 and 95% air). After staining with Giemsa's solution, the mean number of latex particles ingested per macrophage was determined by microscopy. Macrophage H_2O_2 release was assayed as described in the previous paper[17]: Oxidation rate of scopoletin was measured in the incubation medium (2.5 ml) containing 5 nmoles of sco-

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poletin and 125 μg of horseradish peroxidase in Hanks' balanced salt solution (+0.1% glucose) with or without 10 ng/ml of phorbol myristate acetate (PMA) or 4 μg/ml of M protein, which was overlaid on the macrophage monolayer (about 10^6 cells). Macrophage O_2^- release was assayed by the method of Johnston et al.[7] Briefly, macrophage monolayer was overlaid with 2.5 ml of Hanks' balanced salt solution (+0.1% glucose) containing 80 μM cytochrome C (Sigma) with or without addition of either M protein or PMA. The reduction of cytochrome C at 37°C for 90 min in a CO_2 incubator was measured spectrophotometrically. Effect of M protein on macrophage-mediated host resistance against listerial infection was examined as follows: C3H/HeN or NIH Swiss (inbred) mice 7 to 8 weeks old were treated intraperitoneally (ip) with 0.1 to 20 μg of M protein before and/or after intravenous infection with *Listeria monocytogenes* EGD (10^4 or 10^6), and the colony forming units (CFU) in the liver and spleen were determined on trypto-soy agar plates.

When mice (male C3H/HeN, 8 to 10 weeks old) were given ip ovalbumin (20 μg), M protein (20 μg), or OK-432 (0.1 mg), the number of the peritoneal exudate cells were 4.9×10^6, 5.4×10^6, and 2.6×10^7 per mouse, respectively, 4 days after administration. Because the number of peritoneal cells from normal mice was 4.3×10^6 per mouse, M protein, different from OK-432, seems not to induce accumulation of macrophages in its injection site. Thus, M protein at this dose (20 μg per mouse) seems not to elicit chronic inflammation. In this respect, M protein differs from accumulating irritants of macrophage such as casein, thioglycollate, lipopolysaccharide and PPD. Fig. 1 shows the phagocytic abilities of macrophages elicited with M protein, OK-432, or ovalbumin. The phagocytic abilities of M protein as well as ovalbumin-elicited macrophages were 1.5 times higher than those of the resident macrophages. This indicates that these proteins stimulate the macrophage phagocytic function to some degree.

M protein-elicited macrophages showed a significant responsiveness to PMA in respects to

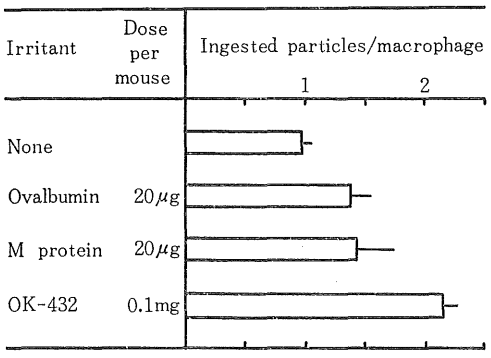

Fig. 1. Comparison of macrophages induced with ovalbumin, M protein, and OK-432 for their phagocytic abilities against latex particles. The macrophages elicited by ip injection of each irritant 4 days before harvest and resident macrophages were tested for their phagocytosis of latex particles as described in the text.

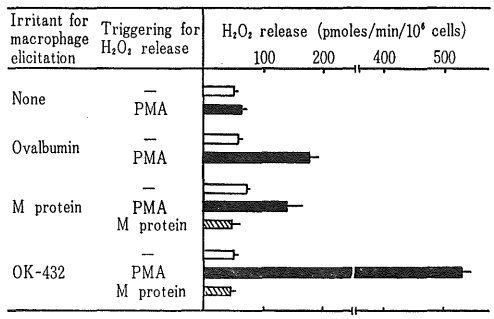

Fig. 2. PMA-triggered H_2O_2 release from macrophages induced by ip injection of ovalbumin (20 μg), M protein (20 μg), or OK-432 (0.1 mg) 4 days before harvest and taken from normal macrophages. Triggering of H_2O_2 release was achieved by the addition of 10 ng of PMA per ml or 4 μg of M protein per ml.

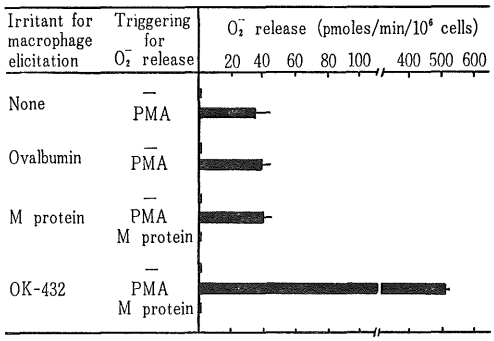

Fig. 3. PMA-triggered O_2^- release from macrophages induced by ovalbumin, M protein, and OK-432 or resident macrophages. Elicitation of macrophages with irritants and triggering of O_2^- release were the sama as described in the legend to Fig. 2.

H_2O_2 (Fig. 2) and O_2^- (Fig. 3). Similar results were obtained in the case of ovalbumin-elicited macrophages. PMA-responsiveness of OK-432-elicited macrophages was much higher than that of the other two. M protein itself did not exhibit any triggering action agaist H_2O_2 and O_2^- releases of macrophages.

Table 1 shows the effect of M protein on the resistance to listerial infection in mice, when given 2 hr after infection. The CFU in the liver 3 days after infection did not decrease by intraperitoneal administration of M protein at doses of 0.1 to 10 μg per mouse. Table 2 shows the effect of double injections of M protein on resistance to infection when administered before (−7 or −2 days) and after (2 hr) listerial infection. M protein, however failed to enhance the resistance to listerial infection even by double injections. As reported earlier[3,10-12], host resistance to infection with *Listeria monocytogenes* is mainly mediated by fixed and free macrophages such as Kupffer cells and blood monocytes newly emigrated to the infection sites. The present findings may, thus, indicate that M protein has no ability to enhance the macrophage-mediated antimicrobial functions. The ability of phagocytic cells to produce active oxygens such as O_2^-, H_2O_2, ·OH, and $^1O_2$ is known to correlate well with their antimicrobial activities[6]. M protein did not trigger H_2O_2 and O_2^- releasing functions of macrophages, but increased slightly the responsiveness of macrophages to triggering of oxidative bursts by PMA.

Table 1. Effect of M protein on the host resistance against listerial infection[a]

Dose of M protein [b] (μg/mouse)	Number of *Listeria* in the liver (log[CFU/organ])
0	3.51±0.28 [c]
0.1	3.49±0.18
1	3.36±0.03
5	3.53±0.07
10	3.47±0.01

[a] NIH Swiss mice were infected iv with 1×10^4 of *L. moncytogenes* and then given M protein. CFU in the liver of the animals were determined 3 days after infection.
[b] M protein was given to mice *via* the intraperitoneal route 2 hr after infection.
[c] The mean ± SE (n=3).

Table 2. Effect of double injections of M protein on the host resistance against listerial infection [a]

Protocol of M protein injection [b]	Number of *Listeria* (log [CFU/organ])	
	Liver	Spleen
None injected	8.68±0.07 [c]	7.65±0.15
2 hr after infection	8.66±0.14	7.49±0.25
2 days before infection	8.67±0.08	7.59±0.22
2 days before and 2 hr after infection	8.66±0.08	7.61±0.14
7 days before and 2 hr after infection	8.76±0.09	7.68±0.06

[a] C3H/HeN mice were infected iv with 1×10^6 of *L. monocytogenes*, and CFU in the liver and spleen of the animals were determined 3 days after infection.
[b] M protein was injected ip to mice at the dose of 20 μg per mouse.
[c] The mean ± SE (n=3).

In summary, the present study indicates that M protein does not modulate macrophage functions related to antimicrobial capacity, and that macrophages have no receptor site specific for M protein in the triggering action on H_2O_2 and O_2^- release of macrophages. In this respect, mouse peritoneal macrophages differ from human lymprocytes: the latter responds to M protein resulting in blastogenesis[15,16], suggesting the existence of some specific reseptor sites for M protein on these cells. However, as reported by Hirata and Terasaki[5], the proliferative response of human lymphocytes to M protein may merely reflects the phenomenon that the alloreactive T cells responded to the HLA antigenic determinants shared by M protein.

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