The Role of Complement Receptors in Tumor Cell Destruction

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OKUDA, T. and TACHIBANA, T. *The Role of Complement Receptors in Tumor Cell Destruction.* Tohoku J. Exp. Med., 1992, **168** (2), 417-420 — Murine peritoneal macrophages exuding early after stimulation with the activators of the alternative complement pathway are able to destroy some of the tumor cells. This destruction is inhibited by anti-Mac-1 and anti-C3. The tumor cell killing needs longer incubation time (>15 hr), and anti-Mac-1 affects the reaction even if it were put into the reaction mixture at later time of incubation. The results suggest that complement produced by macrophage is deposited onto target cells, and the complement binding targets interact with macrophages through the complement receptor type 3 (CR3), which leads to the cell destruction. It is known that fully activated macrophages kill the tumor cells with the aid of antibody (ADCC) or lectin, but we show here another route of tumor cell destruction, that is, some sort of incompletely activated macrophages can kill some type of tumor cells in cooperation with endogenous complement and complement receptors (CR3) without participation of antibody. ——— Mac-1; complement C3; cytolysis

The third component of complements has multiple functions, and there exist elegant and strictly regulated mechanisms controlling these functions in the body. Immunocompetent cells have receptors for various activated C3 fragments, i.e., C3a receptors, CR1, CR2, CR3 and CR4. Macrophages synthesize and secrete various amounts of the complement components and their regulatory proteins according to their states, moreover, they express complement receptors CR1 and CR3 on their surface. These facts prompted us to examine the possibility that complement receptors on macrophages and complement produced by themselves are responsible for some extent for macrophage function at the sites of reaction. This paper describes the involvement of C3 produced in situ by macrophages and of their CR3 in tumor cell's killing.

MATERIALS AND METHODS

Macrophages were obtained from peritoneal exudate cells (PEC) of C3H/HeSlc mice injected with 0.1 mg of OK432 (Streptococcal preparation, Chugai Pharmaceutical Co., Ltd., Tokyo) one day before the harvest. They were put into microplate, and after incubation for 90 min in a CO₂ incubator, the monolayers were washed vigorously to remove nonadher-

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ent cells. Serum free RPMI 1640 containing 1/10 volume of hybridoma culture supernatants or appropriate dilutions of purified antibodies and target cells labeled with ^{125}I -UdR were added. The released radioactivity was determined after 21-24 hr of incubation.

RESULTS

YAC-1 cells were lysed dose-dependently by the adherent peritoneal exudate cells elicited with OK-432. The lysis was inhibited in the presence of 1/10 volume of anti-Mac-1 culture supernatant, but not in the presence of anti-Mac-2, anti-I-A^k, anti-LFA-1 or anti-L3T4 (Fig. 1). The lysis could be scarcely observed after five hours, and it took more than 15 hr to complete the reaction (Fig. 2). F(ab')₂ anti-Mac-1 also inhibited the lysis dose dependently, although the control

Fig. 1. Effect of various antibodies on killing of YAC-1 cells by peritoneal macrophages.

Fig. 2. Effect of adding anti-Mac-1 at different time points. Control medium (○) or anti-Mac-1 (●) were added at the time indicated, then the release at 24 hr was estimated.

Fig. 3. Effect of anti-C3^{mouse} on the lysis of YAC-1.

- (A) F(ab')₂ goat anti-C3. Antibody concentrations are 2 µg/ml (●), 8 µg/ml (○), 20 µg/ml (▲), and 80 µg/ml (△).
 (B) F(ab')₂ goat anti-IgG^{mouse}. ●, 10 µg/ml; ○, 33 µg/ml; ▲, 100 µg/ml.
 (C) F(ab')₂ fragment of normal goat IgG. ▲, 5.5 µg/ml; ●, 18 µg/ml; ○, 55 µg/ml.

F(ab')₂ of normal rat IgG did not. Thus, it is evident that the lysis of YAC-1 by PEC is specifically inhibited by anti-Mac-1. The investigation of the effects of goat F(ab')₂ anti-C3^{mouse} and anti-IgG^{mouse}, or of normal IgG revealed that only anti-C3^{mouse} inhibited the lysis dose-dependently (Fig. 3). Other tumor cells were examined by the same way for their susceptibility to lysis by adherent PEC. RL σ 1 (radiation induced thymoma cells), Meth A and RDM4 (T cell leukemia cell line) were susceptible and their lysis were inhibited by anti-Mac-1. EL4, MM48 (C3H mammary tumor cells) or P815 — known to be susceptible target cells to the activated macrophages — were resistant. Because the target spectrum suggested NK cells as the effector cells, the effect of anti-Mac-1 on NK cell activity of G10-passed normal spleen cells was examined, and it showed that anti-Mac-1 had no effect on NK activity. Using cells purified by discontinuous density gradient centrifugation, the effector cells are proved to be macrophages and not neutrophils. Also other activating substances than OK432 were studied, and Lentinan (β -1, 3 glucan isolated from an edible mushroom, *lentinus edodes*, (Chihara et al. 1969)) and TAK (a gel-forming-1, 3 glucan) were found to be as effective as OK432. All these substances are known as activators of the alternative complement pathway (Okuda et al. 1972; Matsushita et al. 1983).

We showed here that the peritoneal cells exuding early after i.p. injection of OK432, Lentinan or TAK have the ability to kill some tumour cells. This killing reaction was specifically inhibited by anti-Mac-1 and anti-C3. It is reported that there are two steps of activation of macrophages to perform tumour cell killing. The killing reaction by fully activated macrophages was not inhibited by anti-Mac-1 (data not shown). The effector macrophages reported here seem to be different from the fully activated macrophages because they are generated early

after stimulation, and the target spectrum is not that of the fully activated macrophages. From these data it is possible to conclude that some macrophages generated early by stimulation with complement activating substances have some tumour cell's killing ability in cooperation with Mac-1 antigen.

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