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Murine T cell proliferation can be specifically augmented by macrophages fed with specific antigen: α-2-macroglobulin conjugate

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SUMMARY : Foreign antigens conjugated to α-2-Macroglobulin (α-2-M) were effectively taken up by murine macrophages via α-2-M receptors. Such effective internalization of α-2-M:antigen conjugate by macrophages resulted in a remarkable increase in its ability to activate murine immune T cells under the following conditions. After macrophages were incubated with α-2-M:antigen conjugate or unconjugated antigen, they were cultured with immune T cells and antigen-stimulated tritiated thymidine incorporation by T cells was measured. The stimulation of T cell proliferative response by macrophages fed with the conjugate was sixteen times higher than what was observed with macrophages pretreated in the same concentration of unconjugated antigen. These findings suggest a physiological function of α-2-M and give us a new technique of immunization.

Human α-2-M is a 725,000 molecular weight plasma glycoprotein composed of four identical polypeptide chains(1-3). α-2-M inhibits many types of endoproteinases from all four major categories through a unique mechanism called "trapping" hypothesis(4). When α-2-M inhibits proteinases, it undergoes a conformational change with the cleavage of internal thiol ester bonds. Lysine side chains on inhibited proteinases are known to form cross-links with glutamic acid side chains derived from thiol ester bonds(5,6). During the reaction of α-2-M with

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proteinases, α-2-M can bind coexisting proteins other than proteinases(7,8). The conformational change of α-2-M with proteinases results in an exposure of α-2-M receptor recognition site(9). The receptor recognition site on α-2-M molecule is conservative between human and mice(10,11).

We have recently reported a new and useful method to inject into fibroblasts certain enzymes as conjugates with α-2-M via α-2-M receptors on the cell surface(8,12). In this report we focus on the fact that α-2-M receptors exist on the surface of macrophages and tried to enhance the function of macrophages. In the early stage of immune response, macrophages are considered to play an important role as antigen presenting cells(13). It is generally accepted that macrophages take up antigens, handle them and present them to T cells. Here, we report that antigen uptake and antigen presentation to T cells by macrophages is effectively achieved by using α-2-M:antigen conjugates.

MATERIAL AND METHODS

Proteins.

α-galactosidase from coffee beans was purchased from Sigma Chem. Co.(St. Louis, MO) and purified with HPLC gel chromatography(TSK G3000 SW). α-2-M: α-galactosidase conjugate was prepared as described previously(8). To be used in antigen uptake studies, α-galactosidase was labeled with iodine-125 according to the method described previously(14).

Antigen uptake by macrophage.

$P388D_1$ cells (macrophage cell line) were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FCS). For antigen uptake study, $P388D_1$ cells were grown to 50% confluency in 35 mm dishes and the cell monolayers were washed with DMEM containing 5mg/ml bovine serum albumin(DMEM-BSA). The medium was replaced with 1 ml of DMEM-BSA containing ^{125}I-antigen: α-2-M conjugate or the mixture of ^{125}I-antigen and trypsin-reacted α-2-M. The cells were incubated for 2 h and the cell monolayers were washed three times with phosphate buffered saline. The cells were then solubilized with 0.3 ml of 0.1 N NaOH at 37°C for 30 min and radioactivity was measured in a γ-counter. The protein concentration of the cells was determined by a Bio Rad Protein Assay kit(Bio-Rad, U.S.A.).

Assay for the antigen presenting activity of macrophage.

Antigen presenting activity of macrophage was determined with T cell proliferative response in the following system. $P388D_1$ cells were plated in a 96-well tissue culture plate(Falcon

Labware, Oxnard, CA) at $2x10^4$ cells/well in DMEM supplemented with 10 % FCS. After 24h incubation, the cell monolayers were washed twice with DMEM-BSA and the medium was replaced by 0.2ml of DMEM-BSA containing antigen:α-2-M conjugate or unconjugated antigen. After 3 h, mitomycin C at 30 μg/ml was added to each well and the cells were incubated for 30 min at 37°C. The cell monolayers were then washed four times with DMEM-BSA and T cells were added to each well at $3x10^5$ cells/well. T cells were obtained from the spleen of BALB/c mice with Sephadex G-10 column(15) and nylon fiber column(16). Erythrocytes were removed by ammonium chloride lysis before spleen cells were passed through the columns. BALB/c mice were immunized with 20 μg α-galactosidase emulsified in Freund's complete adjuvant two weeks before. Antigen-pulsed $P388D_1$ and T cells were cultured for 4 days in RPMI 1640 medium supplemented with 10 % FCS. Twenty hours before the harvesting of the cells, 0.5 μCi of tritiated thymidine(25 Ci/mmol, New England Nuclear Corp.) was added to each well. The cells were then harvested and collected on glass fiber filters(GF/C Whatman). The filters were washed with 10% ice-cold trichloroacetic acid and the radioactivity on the filter was counted with a liquid scintillation counter.

RESULTS AND DISCUSSION

The $P388D_1$ macrophage cell line was used to study antigen uptake and α-galactosidase from coffee beans was used as foreign antigen. The foreign antigen conjugated to α-2-M was taken up by $P388D_1$ cells as effectively as by human fibroblasts(8,12). The dose-dependency of antigen uptake into $P388D_1$ cells is given in Fig.1. $P388D_1$ cells were incubated for 2 h in DMEM-BSA containing varying concentrations of antigen: α-2-M conjugate or unconjugated antigen. When $P388D_1$ cells were incubated with DMEM-BSA containing 60 μg/ml antigen: α-2-M conjugate, 0.096 μg of antigen per mg of cell protein was internalized into the cells. To achieve the same amount of antigen uptake with unconjugated antigen, the cells had to be incubated with about 110 μg/ml of unconjugated antigen. As 60 μg of the conjugate contained about 3 μg antigen in this case, efficiency of antigen uptake with the conjugate was 37 times higher than that with unconjugated antigen.

Next we studied whether the efficiency of internalization of antigen conjugated to α-2-M was accompanied by the stimulation of antigen specific T cell proliferation or not. T cells were

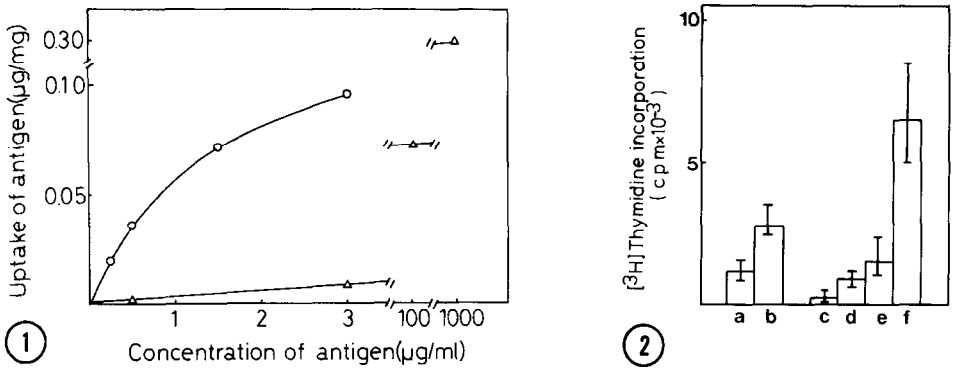

Fig.1. Dose-dependency of the internalization of α-2-M:antigen conjugate(-O-) and free antigen(-Δ-). The numbers on the abscissa indicate concentrations of antigen. 20 μg of the conjugate contained about 1 μg of antigen. Incorporation of antigen into P388D$_1$ cells was represented on the ordinate as μg of antigen per mg of cell protein. The specific activity of ^{125}I-antigen was 1x10^5 cpm/μg.

Fig.2. Antigen specific proliferative response of T cells. Before T cells were added to each well, P388D$_1$ cells were incubated with antigen:α-2-M conjugate or with free antigen in the following concentrations. (a) and (b) : 20 and 100 μg/ml of conjugate, respectively. (c), (d), (e) and (f) : 5, 10, 100 and 1000 μg/ml of free antigen, respectively in the presence of 100μg/ml trypsin-reacted α-2-M. In terms of equivalent antigen concentration, the result of (b) should be compared with that of (c). Tritiated thymidine incorporation into DNA was represented as cpm in cultures containing P388D$_1$ cells that had been incubated with antigen minus cpm in cultures containing P388D$_1$ cells that had been incubated with no antigen. Data include the results of 6 series of T cell activation experiments.

isolated from the spleen of BALB/c mice immunized with 20 μg α-galactosidase with Freund's complete adjuvant two weeks before. Prior to incubation with T cells, P388D$_1$ cells were pulsed with antigen:α-2-M conjugate or unconjugated antigen. First, tritiated thymidine incorporation by T cells in response to free antigen stimulation was studied. With increasing concentrations of antigen, activation of T cells by P388D$_1$ cells increased as given in Fig.2(c,d,e,f). Second, P388D$_1$ cells were incubated with 100 μg/ml of antigen:α-2-M conjugate. The incorporation of tritiated thymidine into DNA of T cells in this case reached 2700 cpm. The stimulation of T cells with the conjugate at concentration of 100 μg/ml was 16 times higher than what was observed with the equivalent concentration of

unconjugated antigen(5μg/ml). In conclusion, the effective internalization of antigen:α-2-M conjugate by macrophages led to a marked increase in the proliferative response of immune T cells primed with the same antigen.

This finding suggests a physiological function of α-2-M. In the case of viral infection, lysis of the infected cells usually occurrs and a large number of free viruses and virus related substances are released along with the intracellular proteinases. In the case of bacterial infection, inflammation such as tissue injury occurrs with the release of a large amount of proteinases. Under these conditions, α-2-M is expected to be on hand to inhibit such proteinases. Here we expect that foreign antigens derived from viruses or bacteria would be conjugated with α-2-M by the action of proteinases. The high concentration of α-2-M in plasma(2-4 mg/ml) is advantageous for the formation of antigen:α-2-M conjugate. If α-2-M can bind foreign antigens, it also has a chance to bind self-proteins. But this poses no problem in the selective immune response because phagocytosis by macrophage is nonspecific at any rate and the distinction between self- and nonself-proteins should be effected later by T cell receptors. Our study also suggests a new immunization technique which depends on an effecient uptake of α-2-M:antigen conjugate by macrophages.

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