Novel rosette formation of murine spleen cells with autologous red blood cells

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Abstract—Spleen cells of normal BALB/c mice formed rosettes with autologous red blood cells, and the formation was calcium ion dependent. Peritoneal exudate cells, bone marrow cells and thymocytes did not form such rosettes. Spleen cells were passed over a Sephadex G-10 column or incubated on a plastic surface in order to eliminate adherent cells from them. Cells obtained by both these methods were unable to form rosettes. B cell-, T cell- and natural killer cell-enriched fractions in spleen cells were unable to form rosettes either. Some of the mouse IgG subclasses suppressed rosette formation when added to its site. These are monoclonal antibodies whose specificities are directed against Aspergillus niger glucose oxidase. Moreover, Aspergillus niger glucose oxidase suppressed the rosette formation when spleen cells had been treated with it as well as when it had been added to the site of rosette formation. These findings suggest that some murine spleen cells have receptors to a structure on autologous red blood cells, which is recognized by an anti-Aspergillus niger glucose oxidase monoclonal antibody.

Key words: Rosette; spleen cells; red blood cell; anti-Aspergillus niger glucose oxidase antibody.

INTRODUCTION

Rosette formation with autologous red blood cells (RBCs) has been amply reported in mice [1–5]. These reports have indicated rosette formation mainly by T cells with autologous or allogeneic RBCs. There are as yet no published data on rosette formation by adherent cells with autologous RBCs. However we have now observed rosette formation of adherent cells with autologous RBCs in the spleen. In order to elucidate this novel type rosette formation, we proposed two possibilities. One possibility was that receptors for the Fc portion of the IgG molecules on splenic macrophages bind IgG on senescent autologous RBCs. There are numerous reports that senescent RBCs bind IgG autoantibodies in both humans [6–8] and animals [9]. The other possibility was that lectins on splenic macrophages bind sugars on autologous RBCs. Crocker and Gordon [10] reported that receptors for sheep
RBCs on resident murine bone marrow macrophages are lectin-like agglutinin with specificity for sialylated components on sheep RBCs. In this report, characteristics of this type of rosette formation will be described.

MATERIALS AND METHODS

Animals

Five to seven-week-old female BALB/c mice were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan).

Cell preparation

Bone marrow cells were prepared from the femora of mice. Briefly, femora were cut at both ends, and marrow cells were flushed out by injecting 4 ml of modified Eagle’s minimum essential medium (MEM) (Nissui Seiyaku Inc., Tokyo, Japan). Modified MEM was supplemented with 2 mM L-glutamine, 25 mM HEPES (Wako Pure Chemical Industries, Osaka, Japan) and 5 × 10⁻⁵ M 2-mercaptoethanol. The cell suspension thus formed was passed through a fine stainless mesh. Spleen cells or thymus cells were prepared by teasing the spleen or thymus in modified MEM and by passing the formed cell suspension through a fine stainless mesh. Peritoneal exudate cells were prepared by lavaging the peritoneal cavity of the mouse, which had been injected with 1.5 ml of 3% thioglycollate medium (Difco Laboratories, Detroit, Michigan, USA) intraperitoneally 4 days before, with phosphate buffered saline (PBS). All cells were exposed to distilled water for 30 s followed by addition of an equal volume of 2-fold concentrated PBS in order to remove RBCs [11]. Whole blood was harvested by cardiopuncture using heparin as an anticoagulant and washed 4 times with modified MEM. The packed cells were resuspended at various concentrations and used as RBCs.

Rosette formation

Lymphoid cells (1 × 10⁶) were incubated with autologous RBCs (1.6 × 10⁷) in 0.2 ml modified MEM containing 10% fetal bovine serum (FBS) (ultra-low IgG, Gibco, NY, USA) at 4°C for 15 or 30 min. The cell suspension was centrifuged at 150 g for 5 min at 4°C followed by gentle agitation. The number of rosettes in 7 μl of the cell suspension was counted microscopically. In another experiment where we attempted to observe the fate of rosettes with an inverted microscope, the cell suspension containing rosettes was diluted with 10% FBS modified MEM so that one or two rosettes existed in a well of the Terasaki plate (Sumitomo Bakelite Co., Ltd., Tokyo, Japan) which contained 15 μl of the medium and was incubated at 37°C for varying periods in a 5% CO₂ water-saturated atmosphere.