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A DISCUSSION OF ANTI-ASPERGILLUS NIGER GLUCOSE OXIDASE MONOCLONAL ANTIBODY REACTIVITY TO RED BLOOD CELLS OF SEVERAL SPECIES

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ABSTRACT

We observed that mouse spleen cells form rosettes with autologous red blood cells (RBCs) and that rosette-formation was suppressed by anti-*Aspergillus niger* glucose oxidase monoclonal antibody (mAb).[1)] In the present study, we investigated whether RBCs of species besides mice have the structure recognized by anti-*A. niger* glucose oxidase mAb by using rosette-formation and complement-mediated hemolysis. Lysates of monkey and human RBCs did not suppress rosette-formation whereas autologous (mouse), rat and sheep RBC lysates partially suppressed rosette-formation. Those lysates exerted their suppressive activity after they had been treated at 56°C for 30 min. *A. niger* glucose oxidase also suppressed rosette-formation with or without treatment at 56°C for 30 min. Alternatively, anti-*A. niger* glucose oxidase mAb lysed mouse, rat and sheep RBCs but not human RBCs with complement. These findings suggest that the cell surfaces of mouse, rat and sheep RBCs have a structure which can be recognized by anti-*A. niger* glucose oxidase mAb while the cell surfaces of monkey and human RBCs do not.

Key Words: Glucose oxidase, Red blood cells

INTRODUCTION

We observed a rosette-formation of mouse spleen adherent cells with autologous RBCs, and suppression of this rosette-formation when autologous RBCs but not spleen cells were incubated with anti-*Aspergillus niger* glucose oxidase monoclonal antibody (mAb) in advance. *A. niger* glucose oxidase also suppressed rosette-formation when spleen cells were incubated with *A. niger* glucose oxidase in advance.[1)] Generally, anti-*A. niger* glucose oxidase mAb is used as a control mAb in immunohistochemical staining of mammalian tissues since mammalian tissues have been thought to have no *A. niger* glucose oxidase.[2)] We have currently proposed a hypothesis that mouse RBCs have a common structure with *A. niger* glucose oxidase and that mouse spleen adherent cells have binding sites which recognize this structure on their surfaces. In the present study, we investigated whether this phenomenon exists in other species using two main methods: rosette-formation and complement-mediated hemolysis.

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MATERIALS AND METHODS

RBCs and their lysates

Whole blood of mice (female Balb/c), rats (female Donryu), sheep, monkeys (Macaca rhesus) and humans were used as RBCs after being washed with normal saline 3 times. Sheep and monkey RBCs were purchased from Nippon Seibutsu Zairyou Center Inc. (Tokyo, Japan). RBC lysates were prepared either by freezing and thawing RBCs 5 times in modified MEM (Eagle's minimum essential medium supplemented with 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol and 25 mM HEPES) (Table 1), or by exposing RBCs to hypotonic shock using distilled water and 2-fold concentrated phosphate-buffered saline[3] (Table 2).

Spleen cell preparation

Mouse spleen cells were prepared by teasing the spleen in modified MEM. Whole spleen cells depleted of RBCs by hypotonic shock were used as rosette-forming cells.

Table 1 Suppression of the rosette-formation of mouse spleen cells with autologous RBCs by various specific RBC lysates

Experiment	Lysate of RBCs	The number of rosettes/ 3.5×10^4 spleen cells[a]
1	–	75±11
	Mouse	40± 5
	Monkey	69± 5
	Human[b]	115± 6
2	–	76± 5
	Mouse	32± 5
	Rat	30± 4
	Sheep	30± 4

[a] Results are expressed as the mean ± standard error of 3 experiments.
[b] Blood type: 0.

Table 2 Effect of heating murine or sheep RBC lysate on its suppressive activity regarding rosette-formation by murine spleen cells with autologous RBCs

Added lysate	The number of rosettes/ 3.5×10^4 spleen cells[a]
None	22.3±2.9
Intact autologous RBC	7.7±2.0
Heated autologous RBC	7.8±2.5
Intact sheep RBC	10.5±2.3
Heated sheep RBC	6.7±2.4
Intact *A.niger* glucose oxidase	8.2±3.2
Heated *A.niger* glucose oxidase	9.0±2.9

[a] Results are expressed as the mean ± standard error of 3 experiments.

Inhibition of rosette-formation by RBC lysates

Mouse spleen cells (1×10^6) were incubated with a RBC lysate (derived from 3.2×10^7 RBCs) at 4°C for 30 min in 0.2 ml of 10% fetal bovine serum (FBS) (ultra-low IgG, GIBCO, NY, USA) modified MEM. After being washed twice with modified MEM, those spleen cells were incubated with autologous RBCs (1.6×10^7) at 4°C for 15 min in 0.2 ml of 10% FBS modified MEM. The cell suspension was centrifuged at 150 g for 5 min followed by gentle agitation. The number of rosettes in 7 microliters of the suspension was counted with an improved Neubauer hemocytometer (Erma, Tokyo, Japan) microscopically.

Complement-mediated hemolysis

Radioactive sodium chromate (^{51}Cr)-labelled RBCs (5×10^6) were incubated in 0.2 ml of modified MEM with or without anti-*A. niger* glucose oxidase mAb (500 ng/ml) in a tube (Falcon 2054) at 4°C for 45 min. After centrifuging each tube at 1500 rpm for 5 min, the formed pellet was resuspended in 1 ml of modified MEM with or without complement in mouse, rat or human RBCs, the standard complement was used; in sheep RBCs, the low-tox complement was used (final dilution 1:10) and 0.6 ml of the 1 ml was dispensed to 3 wells (0.2 ml/well) in a 96-well round-bottomed microtiter plate. After incubation at 37°C for 60 min, the plate was centrifuged at 1500 rpm for 5 min and the radioactivity of the supernatant (0.1 ml/well) was counted with a gamma counter. Percent release was calculated as follows. % release = (cpm in 0.1 ml of the supernatant) $\times$ 2 / cpm of 1×10^6 of the labelled RBCs ($\times$ 100).

Reagents

A. niger glucose oxidase (type 7s) was purchased from Sigma Chemical Co., St. Louis, MO, USA. Anti-*A. niger* glucose oxidase mAb (mouse IgM) was purchased from DAKO A/S, Denmark. Guinea pig complement (standard and low-tox) were purchased from Cedarlane Laboratories (Hornby, Ontario, Canada).

Statistical analysis

Statistical analysis was performed using Student's t-test.

RESULTS

The effect of various specific RBC lysates on rosette-formation

Autologous (mouse), rat and sheep RBC lysates partially suppressed rosette-formation, although monkey and human RBC lysates did not inhibit the rosette-formation (Table 1).

The effect of heating murine and sheep RBC lysates on their rosette-formation suppression

The lysates of murine (autologous) and sheep RBCs suppressed rosette-formation. *After* they were treated at 56°C for 30 min, their activity was not lost (Table 2). *A. niger* glucose oxidase and its heated products also suppressed rosette-formation.

Complement-mediated hemolysis of various specific RBCs by anti-A. niger glucose oxidase mAb

As compared with anti-*A. niger* glucose oxidase mAb alone, the mAb plus complement induced a higher % release in mouse, rat and sheep RBCs but not in human RBCs (Fig. 1). RBCs incubated with anti-*A. niger* glucose oxidase mAb alone showed a higher % release than those incubated with the medium or complement in mice and rats.

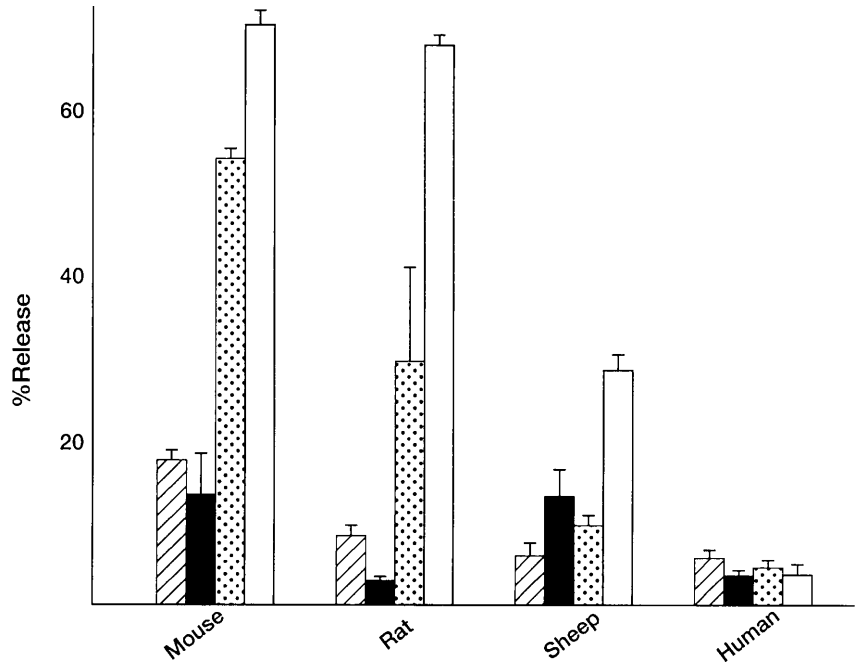


Fig. 1 Complement-mediated hemolysis of RBCs sensitized with anti-*Aspergillus niger* glucose oxidase mAb
Mouse, rat, sheep or human RBCs were incubated with medium alone (▨, ■) or anti-*Aspergillus niger* glucose oxidase mAb (⊡, □) at 4°C for 45 min. Thereafter, those RBCs were incubated with (■, □) or without (▨, ⊡) guinea pig complement at 37°C for 60 min.

DISCUSSION

Mouse spleen cells formed rosettes with rat and sheep RBCs as well as with autologous RBCs (data not shown). However, human RBCs did not form rosettes in the same experiment, which might relate to the size of human RBCs (larger compared with mouse spleen cells). Monkey RBCs formed aggregates without mouse spleen cells. Those aggregates were unable to be discriminated from true rosettes. For these reasons, the competitive inhibition assay with lysates was performed. The rosette-formation of mouse spleen cells with autologous RBCs was suppressed by lysates of autologous, rat and sheep RBCs but not monkey and human RBCs. A. *niger* glucose oxidase also suppressed rosette-formation. After those lysates and A. niger glucose oxidase were treated at 56°C for 30 min, they did not lose their activity to suppress rosette-formation. These findings suggest that there is a common or similar structure on mouse, rat, sheep RBCs and *A. niger* glucose oxidase but not on monkey and human RBCs, and that this structure is comparatively heat-stable. Our previous data showed that the rosette-formation of mouse spleen cells with autologous RBCs is suppressed by anti-*A. niger* glucose oxidase mAb when the RBCs are incubated with the mAb at 4°C.[4] The present study showed that mouse, rat and sheep RBCs but not human RBCs were lysed by anti-*A. niger* glucose oxidase mAb plus complement. In addition, % releases from RBCs (mice and rats) incubated with anti-*A. niger* glucose oxidase mAb only were higher than those incubated without anti-A. *niger*

glucose oxidase mAb. This phenomenon was considered as a spontaneous % release from untrapped (i.e. sensitized) RBCs by anti-A. *niger* glucose oxidase mAb. We observed that sensitized murine RBCs by specific antibody were not trapped by a polystyrene surface and they were transferred to another container (in submission). In that experiment, murine RBCs were sensitized by anti-*A. niger* glucose oxidase mAb (mouse IgG2a) but not anti-keyhole limpet hemocyanin mAb (mouse IgG2a). These findings suggest a possibility that there is a structure recognized by *A. niger* glucose oxidase mAb on mouse, rat and sheep but not on monkey and human RBC surfaces although the structure's physiological significance remains unclear.

REFERENCES

1) Kojima, T., Nagata, H., Tokunaga, Y., Sano, M. and Sasanabe, R.: Novel rosette formation of murine spleen cells with autologous red blood cells. *Haematologia* (in press) (2000).

2) Rathlev, T., Hocko, J.M., Franks, G.F., Suffin, S.C., O'Donnell, C.M. and Porter, D.D.: Glucose oxidase immunoenzyme methodology as a substitute for fluorescence microscopy in the clinical laboratory. *Clin. Chem.*, 27 (9), 1513–1515 (1981).
3) Eremin, O., Coombs, R.R.A., Plumb, D. and Ashby, J.: Characterization of the human natural killer (NK) cell in blood and lymphoid organs. *Int. J. Cancer*, 21, 42–50 (1978).