IMMUNOLOGICAL ACTIVITIES OF MONOCLONAL IgG1 ANTIBODY AGAINST *TRYPANOSOMA GAMBIENSE*

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Abstract. The present paper deals with the immune reaction between a monoclonal IgG1 antibody and *Trypanosoma gambiense*. The aggregation of trypanosomes, immune adherence to macrophages and protection against infection are associated with the antibody. IgG1-mediated clumping of trypanosomes is readily dissociated by the addition of complement. Dissociation of the clumped trypanosomes in the equivalence area released approximately fifty percent of previously bound surface antigens. These antigens were capable of binding again to new IgG1 antibody. Complement deposition rendered bivalent IgG1 antibody in the immune complex functionally univalent. Such an event in the presence of complement is of great advantage to the infected host in killing pathogens *in vivo*, as it allows more antibodies to attach to surface antigens and subsequently to initiate complement activity.

INTRODUCTION

Animals challenged with crude homogenates of blood trypanosomes show protection against the homologous strain of trypanosomes (Osaki, 1959; Weitz, 1960; Seed, 1963; Miller, 1965; Seed and Gam, 1966a; Seed *et al*, 1969). Antiserum passively transferred from immunized donors was also effective in protecting recipients against trypanosomes (Osaki, 1959; Seed, 1963; Seed and Gam, 1966b; Takayanagi and Enriquez, 1973). However, immunologic activities of polyvalent antisera present several problems, because antisera raised against *T. gambiense* contain several classes or subclasses of immunoglobulins, and such antibodies have not been found to be specific for epitopes that reside on the cell surface. A potential solution is to employ the mouse somatic cell hybridization technique. It is now possible to generate clones of hybrid cells that produce predefined antibodies specific for epitopes on the cell surface.

The present paper aims to determine the immunological activities of a mouse monoclonal IgG1 against trypanosomes and attempts to enquire further into the mechanism by which host immune protection is facilitated. It suggests that formation of aggregate masses, promotion of immune adherence to macrophages and protection against trypanosome infection are associated with the IgG1, that the primary bond between trypanosome surface antigen (VSG) and IgG1 antibody is affected when complement fragments are deposited on the immune complexes, and that the covalent binding of complement fragments to the IgG1 antibody appears to render the IgG1 antibody functionally univalent.

MATERIALS AND METHODS

Trypanosomes

A cloned population, comprising a single antigenic type, type O, of *T. gambiense*, Wellcome strain, was used. They were maintained in 18-20g mice (dd strain) by serial transfer at 3-day intervals.

Collection and separation of trypanosomes

Wistar male rats (250 g) were infected intraperitoneally (ip) with 5×10^7 *T. gambiense* from an infected mouse. Blood was collected at peak parasitemia by heart puncture under anesthesia with chloroform. Parasites free of host blood cell components were obtained by means of a DEAE-cellulose column (Lanham and Godfrey, 1970). The parasites were washed 5 times with 1 % glucose phosphate-buffered saline (GPB), ionic strength

0.271, pH 7.5, and centrifuged at 800*g* for 10 minutes. After final centrifugation, the parasites were resuspended in ice-cold GPB.

Cells and culture medium

Myeloma cells (P3-×63Ag8-653) were purchased from Dainihon-Seiyaku Ltd (Tokyo) and maintained in GIT culture medium (Nihon Seiyaku Ltd).

Immunization and hybridization

Six-week-old female BALB/c mice were inoculated ip with 1×10^4 living *T. gambiense* in 0.2ml GPB. Seventy-two hours later, the infected mice were given 0.5 ml of fresh normal human serum ip to completely cure the trypanosome infection. Seventy-two hours after the treatment, splenic lymphocytes were fused with myeloma cells at a ratio of 5/1 in polyethylene glycol 1540 (Katayama Kagaku Ltd, Osaka). Selections of hybridoma cells were carried out using GIT culture medium containing 1×10^{-7}M aminopterin (SIGMA). Screening for the production of antibodies to VSG (variant specific glycoprotein) of *T. gambiense* was done by aggregation reaction as described below. The clone, designated NCU-90, was determined to produce IgG1 antibodies by means of Ouchterlony plate examination. Antiserum to mouse IgG1 was purchased from Cappel Laboratories. This clone was subcloned twice by limiting dilutions and expanded in tissue culture.

Agglutination test

Five-tenths of a milliliter of a 2-fold diluted supernatant were mixed with an equal volume of GPB containing 1×10^8 *T. gambiense*. After 20 minutes at 23°C, the agglutination titers were determined under the microscope (×200) as the highest dilution of supernatant in which agglutination occurred. The titers represented 1:128 as the supernatant dilutions before mixing with an equal volume of antigen suspension.

Determination of the region of equivalence (optimal antigen-antibody ratio)

Two-tenths of a milliliter of the trypanosome suspension at different concentrations were mixed with 0.1 ml of the supernatant (NCU-90). Ten minutes later, the region of equivalence was determined as the highest dilution of trypanosome suspension in which all trypanosomes had clumped (Takayanagi *et al,* 1977). The suspensions containing more trypanosomes than the equivalent suspension were considered as containing antigen excess. The suspensions containing less trypanosomes than the equivalent suspension were considered as containing antibody excess.

Complement depletion of fresh normal rabbit serum

Fresh normal rabbit serum deficient in C3 was prepared by incubation of fresh normal rabbit serum with 10 units of cobra venom factor (CVF) from *Naja naja,* which was purified by DEAE-cellulose column chromatography and Sephadex G-200 gel filtration (Ballow and Cochrane, 1969). The treatment resulted in complete loss of complement hemolytic activity.

Assay of hemolytic activity of treated normal rabbit serum

To assess the hemolytic capacity of the treated normal rabbit serum used, 6×10^6 sheep erythrocytes, optimally sensitized with rabbit hemolysin and suspended in 0.25 ml Mg^{++} gelatin veronal buffer (Mayer 1961), were added to 0.75 ml of the reaction mixtures that contained the same cations, inhibitors, and serum constituents used in the experiments.

Preparation of suspensions of dissociated trypanosomes (D-trypanosomes)

The suspension of trypanosomes aggregated by supernatant NCU-90 at the equivalent antigen-antibody ratio was mixed with fresh normal rabbit serum as a source of complement to completely dissociate the aggregated masses. When dissociation was completed, the suspension was mixed with CVF to inactivate excess complement. The trypanosomes, designated as D-trypanosomes, were centrifuged at 800*g* for 5 minutes. After centrifugation, suspensions containing varying number of the D-trypanosomes in GPB were prepared.

Mouse peritoneal macrophages

The methods used to harvest macrophages from mouse peritoneal cavity and to cultivate

them have been described (Takayanagi *et al*, 1987).

Binding test

The culture medium was removed with a sterile pipette and 0.2 ml of *T. gambiense* suspension was added. After 1 minute, 0.1 ml of supernatant NCU-90 was added. In some experiments, fresh unimmunized rabbit serum (0.2 ml) was added as the source of complement. After mixing thoroughly, the culture tube was incubated at 37°C for 10 minutes and then the coverslip was vigorously washed twice in 2 ml of GPB to remove *T. gambiense* that did not adhere to the macrophages. The coverslip fixed in methanol for 10 minutes and stained in Giemsa was observed under a microscope (× 400).

Passive transfer

Five-tenths of a milliliter of supernatant NCU-90 were injected ip into each recipient mouse (BALB/c, 20 *g* body weight) one hour before trypanosome challenge. Control mice each received an equal volume of P3-x63Ag8-653 myeloma supernatant. To estimate protective ability of the supernatant, trypanosomes (1×10^4) in 0.1 ml GPB were given ip to five recipient mice, which were examined for the presence or absence of the parasites every 24 hours by wet mount. If no parasites were observed by day 10 after infection, then protection was considered to be complete.

RESULTS

Trypanosomes immediately formed aggregates when they came into contact with IgG1 antibodies corresponding to their serotype. Formation of IgG1 antibody-mediated aggregates was affected by the antigen-antibody ratio. Table 1 summarizes the immune reaction of various trypanosome concentrations mixed with the supernatant. Trypanosomes formed aggregates and no free trypanosomes were observed in mixtures I, II and III. These aggregates of trypanosomes formed within a few seconds after mixing the trypanosome suspension with the supernatant. In mixtures IV, V and VI, on the other hand, aggregates of trypanosomes were smaller and the number of free trypanosomes increased as antibody ratio increased. In mixture VII, parasite aggregation was no longer observed in the presence of the supernatant. It was determined from Table 1 that suspension III represented the equivalence point, the point at which the antigen-antibody ratio was optimal. Free trypanosomes in antibody excess regions attached to macrophages, suggesting that the Fc portion of the antibody constituting the complex bound to the macrophage Fc receptor on the cell membrane.

When fresh normal rabbit serum was added to the clumped masses formed at the equivalence point, dissociation of aggregated trypanosomes was readily initiated. A few minutes later, clumped masses were completely dissociated. Dissociated

Table 1

Immune responses in different trypanosome-antibody ratio[1].

Experimental number	Number of trypanosomes ($\times 10^8$/0.2 ml)	Supernatant NCU-90 added (ml)	Clumping	Free trypanosomes (% ± SD)
I	20.0	0.1	+	0
II	10.0	0.1	+	0
III	5.0[2]	0.1	+	0
IV	2.5	0.1	+	20.3 ± 5.7
V	1.3	0.1	+	65.3 ± 6.1
VI	0.16	0.1	+	90.9 ± 8.8
VII	0.08	0.1	−[3]	100

[1] Observations were made 5 minutes later
[2] Equivalence
[3] The reaction did not occur

Table 2

Immune responses between D-trypanosomes and the supernatant NCU-90 in different antigen-antibody ratio[1].

Experimental number	Number of D-trypanosomes ($\times 10^8$/0.2 ml)	Supernatant NCU-90 added (ml)	Clumping	Free D-trypanosomes (% ± SD)
1	20.0	0.1	+	0
2	10.0[2]	0.1	+	0
3	5.0	0.1	+	10.3±2.7
4	2.5	0.1	+	29.6±7.1
5	1.3	0.1	+	88.1±8.0

[1] Observations were made 5 minutes later
[2] Equivalence

trypanosomes were noted to be intact and exhibited vigorous flagellar movement. No lysis of dissociated trypanosomes by complement was observed. As soon as the clumped trypanosomes became free, they attached to macrophages (Fig 1), suggesting that they bound to macrophages through C3 receptors.

The D-trypanosomes were subsequently reaggregated by the new addition of the supernatant after excess complement was inactivated by CVF. Table 2 summarizes the reaggregation of various concentrations of D-trypanosomes upon the new addition of the supernatant. D-trypanosomes formed aggregates with no free D-trypanosomes observed in mixtures 1 and 2. On the other hand, D-trypanosome aggregates in mixtures 3, 4 and 5 were smaller and free D-trypanosomes increased as antibody ratio increased. The data from Table 2 show that the D-trypanosome-new IgG1 antibody ratio was optimal in mixture 2.

Passive transfer of immunity was achieved using supernatant NCU-90, and it was effective for complete protection against trypanosomes. Mice given the supernatant ip were protected against infection with as many as 1×10^4 trypanosomes. No control mice were protected against trypanosome infection. They showed parasitemia on day 2 after infection and died on day 4.

DISCUSSION

Hybridomas generated by fusion of splenocytes taken from BALB/c mice immunized with O type *T. gambiense* produced IgG1 antibodies which were specific for O type surface antigen (VSG). They were associated with *in vitro* immune reactions with trypanosomes such as formation of aggregated masses and adherence of trypanosomes to macrophages. IgG1 antibody passively transferred was effective in protecting recipients against trypanosome infection *in vivo*. This *in vivo* event of protection was initially conjectured from the *in vitro* observations on the immune reaction between the trypanosome, the antiserum and complement. Particularly, dissociation of clumping masses in the presence of complement seemed to be an important immune reaction to eliminate pathogens. This dissociation phenomenon may be essentially the same as the solubilization of aggregates, produced by soluble antigen and antiserum, by complement (Morgan *et al,* 1964; Grant 1968; Miller and Nussengweig, 1975; Czop and Nussenzweig, 1976; Takahashi *et al,* 1977; Casali and Lambert 1979; Schifferli *et al,* 1980). In the clumping reaction under optimal antigen-antibody ratio in the absence of complement, both the two V-domains of each IgG1 antibody appeared to combine with the surface antigens of *T. gambiense* to form immunocomplexes. When complement activated the CH-domain (Edelman 1970; Kehoe and Fougereau 1969; Connel and Porter 1971; Allan and Isliker 1974), changes evidently occurred in the IgG1 antibody, resulting in dissociation. It is possible that bivalent IgG1 antibody became univalent in the presence of complement with only

one V-domain of the antibody continuing to bind with the trypanosome.

Such a phenomenon would involve the release of approximately half of the previously bound antigens. These antigens were subsequently free to bind to new IgG1 antibodies in the absence of complement, resulting in the observed clumping of the D-trypanosomes indicated in Table 2. Thus, the optimal antigen-IgG1 antibody ratio in the absence of complement becomes a condition of extreme antigen excess in the presence of complement.

Trypanosomes may be effectively removed from the circulation of experimental animals with trypanosomiasis by passive transfer of antibodies (Osaki, 1959; Seed, 1963; Seed and Gam, 1966b; Takayanagi and Enriquez, 1973). However, the capacity of antibodies to promote phagocytosis was reported to be diminished when peritoneal cell exudate from animals treated with steroids was employed (Patton 1972). This suggested an important role for macrophages in the clearance of trypanosomes (Dusanic, 1975). In systems lacking complement, immune complexes are reported to adhere to the membrane of various cells possessing Fc receptors by means of the Fc portion of its antibody (Uhr and Phillips, 1966; Rabinovitch, 1967; Jones *et al,* 1972). In the presence of complement, immune complexes are suggested to bind to complement to form an immune complex-C3 conjugate, and the conjugate then attaches to cell membranes possessing receptors for fixed C3 (Nelson, 1953; Ross and Polley, 1974). The macrophage has both the Fc and C3 receptors on its cell membrane (Uhr, 1965; Lay and Nussenzweig, 1968). In the presence of antibody and complement the dissociated trypanosomes are presumed to bind to macrophages through both C3 and Fc receptors thus keeping a higher affinity (Fig 1). This type of binding to the macrophage through C3 receptors is important *in vivo,* because the fixed C3 enhances cytotoxic activity of effector cell against target cell by improving effector cell-target cell contact (Ghebrehiwet *et al,* 1979; Perlmann *et al,* 1981).

The described phenomenon of IgG1-mediated clumping of trypanosomes and subsequent dissociation by complement and immunophagocytosis by macrophages seems to be a very effective means of clearing the infected host of *T. gambiense.*

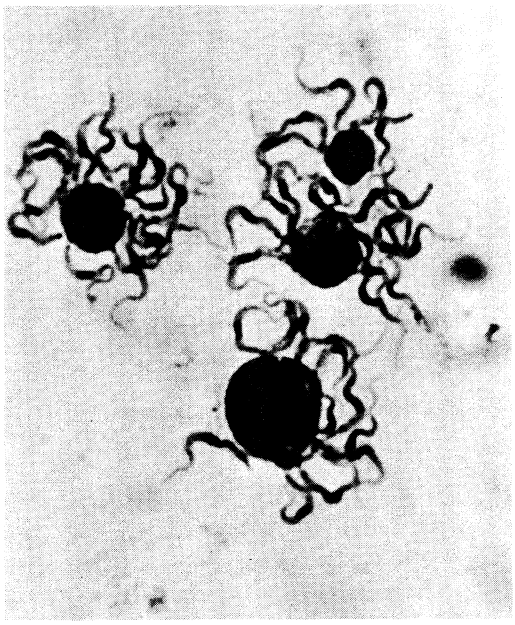

Fig 1—Photograph showing *T. gambiense* dissociated from clumps formed at equivalence by fresh normal rabbit serum, attached to macrophages.

Various biological activities in which antibody is involved are largely due to the biological activities of complement activated by the immune complex. Therefore, the intensity of these biological activities of the antibody is affected by the number of complement molecules activated by the complex. In this study bivalent IgG1 antibody became functionally univalent upon complement activation, with one antibody binding to one antigen on the cell surface of *T. gambiense.* This change to a univalent antibody upon combination with complement may have also caused allosteric change in its Fc portion favoring the binding to the macrophage receptor. *T. gambiense* is known to have a great number of surface antigens, many of which consist of glycoproteins (Cross, 1978). Therefore, a large number of univalent antibodies may be mobilized for the formation of immunocomplexes and subsequent activation of complement, resulting in protection against trypanosome infection.

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