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Monoclonal Antibody to a Major Glycoprotein Immunogen Mediates Differential Complement-Independent Lysis of *Trichomonas vaginalis*

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An immunoglobulin G type 2a (IgG2a) monoclonal antibody (MAb), designated C20A3, which reacts with a highly immunogenic trichomonad membrane glycoprotein (~270,000 daltons), produced complement-independent cytolysis of *Trichomonas vaginalis* organisms. Time- and temperature-dependent lysis of parasites was observed following incubation of washed, live *T. vaginalis* with certain concentrations of C20A3 IgG. Differential killing of trichomonal isolates and clones of a given isolate by C20A3 was dependent on the presence of the glycoprotein antigen on the parasite surface.

Extensive heterogeneity has been reported for *Trichomonas vaginalis*, based on certain immunologic assays (5–7, 9, 12–15). Recent studies indicate that the antigenic distinctions among trichomonal isolates result from the surface disposition of major proteinaceous immunogens (4–7). Differentiation of subpopulations of trichomonads of several isolates was also noted, on the basis of reactions with sera from patients with trichomoniasis (7). Recently, a monoclonal antibody (MAb) toward a major *T. vaginalis* membrane glycoprotein immunogen involved in antigenic heterogeneity was studied (6). Trichomonads without the surface antigen continued to synthesize the glycoprotein. Phenotypic variation of the major surface glycoprotein antigen has also been demonstrated (5). During this earlier study (5), some lysis of trichomonads bearing this surface glycoprotein was occasionally observed under certain circumstances. In this report, we attempt to characterize the properties of MAb-mediated cytolysis of parasites expressing the surface marker. The data show differential complement-independent killing of trichomonal isolates by antibody. These results illustrate the importance of the phenotype of *T. vaginalis* organisms as a possible virulence determinant for this pathogen.

Isolates and clones of *T. vaginalis* used for this investigation have been characterized previously (1, 3–7). Trichomonads were grown in a complex medium of Trypticase (BBL Microbiology Systems)-yeast extract-maltose (TYM) supplemented with heat-inactivated serum (11). Only mid-logarithmic-phase (11) organisms were used for these experiments. Radiolabeling of trichomonads with [^{3}H]thymidine was accomplished as described previously (3). Greater than 95% of cell-associated [^{3}H]thymidine was incorporated into DNA, as determined by 5% trichloroacetic acid precipitation of radioactivity (3).

The immunoglobulin G type 2a (IgG2a) MAb designated C20A3 and monospecific antiserum directed to the trichomonad glycoprotein have been recently characterized (5, 6). An irrelevant IgG2a MAb directed to *Mycoplasma pneumoniae* was used as a negative control. IgG purified by protein A-Sepharose chromatography (Pharmacia, Inc. Piscataway, N.J.) was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) prior to use in outlined experiments. Indirect immunofluorescence and flow cytofluorometric analysis of *T. vaginalis* (5, 6) were performed immediately before using trichomonads in MAb-mediated lysis experiments, as described below. This was done to quantitate trichomonads either positive or negative for fluorescence with the antibody reagents.

To determine MAb-mediated parasite lysis, 0.1 ml of phosphate-buffered saline containing 2×10^6 washed and highly motile unlabeled or ^{3}H-labeled trichomonads was added to siliconized Microfuge (Beckman Instruments, Inc., Fullerton, Calif.) tubes. An equal volume of different concentrations of C20A3 IgG or irrelevant MAb IgG (5, 6) was then added. After incubation at various temperatures for different time periods, intact motile organisms were enumerated by a Neubauer hemacytometer (11). Parasite suspensions were then centrifuged for scintillation spectroscopy of cell-associated versus free radioactivity. Nonmotile trichomonads were not observed under these conditions. All experiments were always repeated at least three times, and each experimental condition included triplicate samples.

Figure 1A shows the time-dependent killing of isolate NYH286 organisms following the addition of 1.2 μg of C20A3 IgG to 2×10^6 washed parasites. Under these conditions, microscopic enumeration of trichomonads revealed that maximal cytolysis occurred by 5 min. Only 85 to 90% of the parasites of isolate NYH286 were killed by C20A3. Higher concentrations of C20A3 IgG or the IgG fraction of monospecific antiserum to the trichomonad antigen did not result in higher levels of parasite killing (Table 1). Surviving trichomonads remained highly motile throughout the experiment. Temperature-dependent cytolysis of trichomonads was also demonstrated (Fig. 1A, inset), and there was less lysis at 4°C than at 22 and 37°C. No death of *T. vaginalis* was ever observed in duplicate samples handled similarly with MAb to *M. pneumoniae*. Also, guinea pig serum added to the reaction mixtures as a source of complement did not affect the overall extent of C20A3-induced cytolysis of NYH286 parasites.

The dramatic and rapid release of radiolabeled DNA from [^{3}H]thymidine-labeled organisms is shown in Fig. 1B. These data paralleled those obtained from microscopic measurements (Fig. 1A). As expected, corresponding increases in radioactivity were detected in the supernatant for the respective time points (data not shown) (3). Finally, an MAb other

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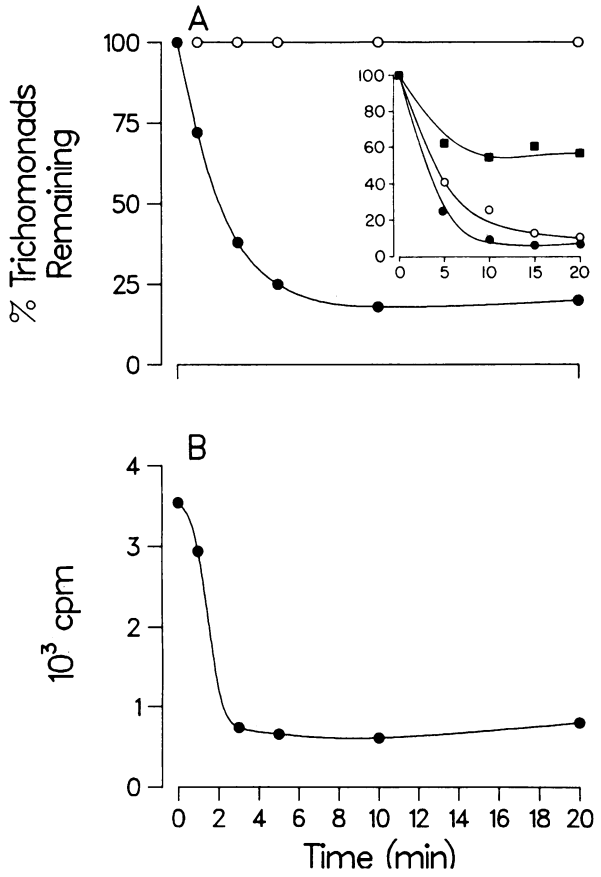

FIG. 1. Time-dependent C20A3 cytolysis of unlabeled (A) or [^{3}H]thymidine-labeled (B) *T. vaginalis* NYH286. Lysis of trichomonads was determined by microscopic enumeration (A) or by measurement of cell-associated or released radioactivity (B) following incubation of 2×10^6 washed parasites suspended in 0.2 ml of phosphate-buffered saline containing 1.2 μg of C20A3 IgG. The effect of temperature on parasite cytolysis caused by the MAb is shown in the inset in panel A.

than C20A3 which recognizes a glycoprotein antigen on all organisms of all isolates that we examined (4–7) (unpublished data) did not lyse trichomonads. These data further confirm the selective complement-independent cytolysis of *T. vaginalis* by C20A3.

We next evaluated the ability of C20A3 IgG to cause cytolysis of several representative trichomonal isolates as well as NYH286 clones (Table 1). In addition, we wanted to establish that only parasites with the trichomonal glycoprotein on their surface were killed (6). Of NYH286 trichomonads of the parent population, 85% were readily killed at antibody levels of ≧1.2 μg (Table 1). The extent of cytolysis corresponded to the percentage of fluorescence-positive parasites (Table 1). This is consistent with the lack of total killing of isolate NYH286 (Fig. 1). Table 1 also shows that trichomonads of isolates RU375 and IR78, which synthesize but do not externalize the surface antigen (6), were unaffected by 10-fold-higher levels of C20A3 IgG. Similar results were obtained for other isolates consisting of heterogeneous staining and nonstaining trichomonads (5, 6).

Finally, Table 1 illustrates the differential killing of two representative agar clones obtained from isolate NYH286. Cytolysis was observed for clones consisting only of positive-phenotype parasites, such as clone 9. The addition of guinea pig serum (as a source of complement) or the use of monospecific antiserum (5) did not result in lysis for trichomonads of clone 3 or isolates RU375 and IR78.

The antigen recognized by the MAb used in this study is a glycoprotein with a molecular weight of 267,000 (6) and is one of a group of surface antigens present in low copy number on *T. vaginalis* organisms (2). Cytolysis occurred only for trichomonads with the antibody-binding antigen on their surface (5, 6) (Table 1). Nonetheless, the ability to perturb trichomonal membranes with C20A3 and possibly antibodies to other antigens is noteworthy. The rapid death and lysis of *T. vaginalis* may indicate important structure-function properties for this surface molecule.

The mechanism of complement-independent, C20A3-mediated cytolysis of pathogenic human trichomonads remains unknown. MAbs, however, have been found to be cytotoxic for other pathogenic protozoa in the absence of complement (8, 10). For example, MAbs to *Trypanosoma cruzi* inhibited parasite metabolism and replication (8). More recently, an MAb to a 170,000-dalton surface antigen of *Giardia lamblia* also produced very rapid killing of trophozoites (10). Interestingly, in both of these examples, the MAbs also differentiated among parasite subpopulations. These data are consistent with the idea that parasites without a major surface immunogen are resistant to antibody-mediated killing. Thus, trichomonads that undergo phase variation during growth and multiplication (5) may be capable of evading humoral immune mechanisms in their host.

TABLE 1. Fluorescence-positive trichomonads and MAb-mediated cytolysis of *T. vaginalis* isolates and clones[a]

Isolate or clone designation	% Fluorescence-positive organisms[b]	IgG[c] protein concn (μg/0.2 ml [final vol])	No. of trichomonads remaining[d]	% Cytotoxicity[f]
NYH286	85	0.012	2.00	0
	85	0.120	0.94	53
	85	1.200	0.31	85
RU375	0	12.0	1.96	2
IR78	≦1	12.0	1.86	7
NYH286, clone 3[e]	0	1.2	2.00	0
NYH286, clone 9	100	1.2	0	100

[a] Approximately 2×10^6 live, motile trichomonads in 0.1 ml were incubated with an equal volume of antibody, as described in the text. Enumeration was performed with a Neubauer counting chamber. Samples handled similarly without antibody or with anti-*M. pneumoniae* MAb of the same isotype did not show lysis of organisms.

[b] Indirect immunofluorescence was performed just before evaluation of parasite lysis, using C20A3 hybridoma supernatant. The percentage of fluorescent organisms was calculated according to the number of fluorescence-positive parasites in at least 10 fields of view on different parts of the slide, as compared with the total number of trichomonads counted by dark-field microscopy of the same fields. Numbers were confirmed by flow cytofluorometric analysis of the same isolates or clones (5). Hybridoma supernatant containing irrelevant MAb was used as a control.

[c] Protein was determined with the Bradford reagent (Bio-Rad Laboratories, Richmond, Calif.).

[d] Data are the means of three separate determinations. Standard deviations never varied by greater than 1% of the mean. The same experiment was repeated at least three times.

[e] Agar clones of trichomonads had defined phenotypes revealed by C20A3 reactivity (7).

[f] Numbers represent the percentage of trichomonads lysed by C20A3.

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