Characterization of monoclonal antibodies to trichocyst antigens in *Paramecium*

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Summary

Ten mouse monoclonal antibodies (mAbs) were raised against trichocyst contaminants present in crude or enriched lysosome fractions of *Paramecium multimicronucleatum*. Using an indirect immunofluorescence assay (IFA) and immunogold labelling on frozen thin sections, epitopes were located on the outer edge, cortex and the core of the trichocyst body, as well as the sheath covering the tip. Except for the two on the tip, epitopes were reactive after SDS–PAGE under non-reducing conditions. Four mAbs (131C1E8, A1-3, A16-2, D7) were directed to a trio of bands of 37, 34 and 29 ($\times 10^3$) M_r from the beaded or meshlike trichocyst body sheath. A fifth mAb (135B9E7), directed to epitopes on the cortex inside the beaded body sheath, reacted strongly with the 37 and 34 bands, but weakly with the $29 \times 10^3 M_r$ band. The last three mAbs (270D5, 22C7F2, D8) were reactive with one or more of three families of antigens found on the trichocyst core. mAb 270D5 reacted mainly with the 34 and 29 ($\times 10^3$) M_r bands of the family containing the above trio, while mAb 22C7F2 reacted consistently with the 47×10^3 band of the higher M_r family but variably with both the trio of bands and the 17×10^3 band of the lower M_r family. mAb D8, which was directed to epitopes on the trichocyst core and small vesicles in the endoplasm, reacted only with the $29 \times 10^3 M_r$ band. The mAbs were cross-reactive with the trichocysts of *P. primaurelia*, *P. tetraurelia*, *P. caudatum* and *P. calkinsi* with some small variation in blotting patterns. Except for the reactivity of mAbs 22C7F2 and 270D5 with a $64 \times 10^3 M_r$ band, *Tetrahymena thermophila* was unreactive with these mAbs either by IFA or after SDS–PAGE and blotting. These results show that: (1) the mAbs obtained are specific for selected polypeptides in four trichocyst locations; (2) antigenic differences exist within the core, cortex and the beaded sheath of the trichocyst body; and (3) the antigenic determinants were conserved in all paramecia species tested.

Key words: cryoultramicrotomy, immunofluorescence, *Paramecium*, trichocyst.

Introduction

Trichocyst development, which has been studied morphologically (Yusa, 1963; Jurand & Selman, 1969), occurs in recognizable steps. The earliest form of trichocyst condensation observed in transmission electron microscopy is the primordial stage, which is recognized by the first indication of a paracrystalline core. This is followed by the pretrichocyst stage, during which the paracrystalline core increases in size, and the immature stage, in which the paracrystalline lattice develops into a body resembling a mature trichocyst. The mature trichocyst, having a body distinguishable by cross-striations of 7 nm periodicity, is fully positioned in the ectoplasm. Extrusion, which can be triggered by numerous factors, such as electrical impulses, pH and temperature changes, occurs within a few milliseconds (Hausmann, 1978; Plattner, 1987). Immediately preceding extrusion, the

trichocyst membrane fuses with the plasma membrane, opening the way for trichocyst release. The extruded proteins are transformed into a needle-like structure with cross-striations of 55 nm periodicity. The bodies of both condensed and extended trichocysts are made up of protein crystals and during discharge the crystal lattice expands (Sperling *et al.* 1987; Peterson *et al.* 1987).

Analyses of extruded trichocyst proteins using SDS–PAGE demonstrated the presence of two proteins of 17 and 36 ($\times 10^3$)M_r (Steers *et al.* 1969; Pollack & Steers, 1973). The latter is reduced to the former after disulphide reduction with 2-mercaptoethanol prior to electrophoresis. These two polypeptides have been shown to consist of closely migrating families of polypeptides of 15–20 and 30–36 ($\times 10^3$)M_r (Adoutte *et al.* 1980; Adoutte, 1988). After high-resolution two-dimensional PAGE, about 50 dominant spots were identified (Tindall, 1986).

Using several polyclonal antibodies prepared against purified extruded trichocyst proteins, Adoutte *et al.* (1984) demonstrated the presence of a third family of proteins having a range of 40–50 ($\times 10^3$) M_r. When cells were treated with monensin, this family of proteins was increased at the expense of the proteins of 15–20 ($\times 10^3$) M_r in the mutant *tam 38*, while in wild-type cells the 40–50 ($\times 10^3$) M_r family was increased but the 15–20 ($\times 10^3$) M_r family was not decreased. In another mutant lacking recognizable mature trichocysts (mutant *tl*) the failure of the paracrystalline structure to develop was accompanied by the absence of the 15–20 ($\times 10^3$) M_r bands. On the basis of these results, it was proposed that the 15–20 and the 40–50 ($\times 10^3$) M_r families represent, respectively, the 'product' and 'precursor' of the trichocyst proteins and that, during maturation, the latter family is proteolytically cleaved to give rise to the former family (Adoutte *et al.* 1984; Adoutte, 1988).

In the course of producing mAbs to use as tools with which to study intracellular digestion in *Paramecium*, more than 10 mAbs were found to react specifically with trichocysts during the initial screening using an indirect immunofluorescence assay. However, before these mAbs could be used to study trichocyst formation or secretion, it was necessary to characterize the M_r of these trichocyst antigens using SDS–PAGE followed by electroblotting, and to verify the location of the epitopes at the ultrastructural level using frozen thin sections. Each mAb was then tested for cross-reactivity with several other species of *Paramecium* and with *Tetrahymena*. The results show that the mAbs were specific for selected polypeptides of three protein families located in the core, the cortex and the beaded sheath of the trichocyst body and that these mAbs cross-reacted with trichocyst antigens in all species of *Paramecium* tested.

Materials and methods

Two strains of *Paramecium multimicronucleatum*, PM$_1$ and PM$_3$ (PM$_3$ was a gift from Dr T. Cole, Wabash College, Crawfordsville, IN), *P. tetraurelia* 51s, *P. primaurelia*, *P. caudatum* and *Tetrahymena thermophila* were each cultured in axenic medium (Fok & Allen, 1979; Fok *et al.* 1987). *P. calkinsi* (a gift from Dr D. Cronkite, Hope College, Holland, MI) was cultured in a mixture of axenic medium, developed for paramecia, and sterile sea water. All cell types were harvested at mid-log phase and prepared according to the procedures described below.

Monoclonal antibodies (mAbs) were produced according to the method of Kohler & Milstein (1975) by the Monoclonal Antibody Center at the University of Hawaii under the supervision of Dr A. A. Benedict. Briefly, Balb/c mice were immunized with crude or enriched lysosomal fractions of PM$_1$. (These fractions were also used as antigens to produce the mAbs specific for the membrane proteins of the phagosome–lysosome system reported by Fok *et al.* (1986).) The spleen cells were then fused with a non-secreting mouse myeloma cell line, P3X63-AG8.653 (Kearney *et al.* 1979). The fused cells were selected for spleen–myeloma hybrids. Hybridomas were screened against fixed and acetone-permeabilized PM$_1$ using an indirect immunofluorescence assay as described (Fok *et al.* 1986). Selected hybridomas were cloned and subcloned before hybridoma supernatant and ascites fluid were collected.

For localization in light microscopy, cells were fixed with 3 % paraformaldehyde in phosphate buffer, pH 7·6, at room temperature. After permeabilization with acetone and washing in phosphate-buffered saline, cells were incubated in mAb hybridoma supernatant followed by a second antibody labelled with fluorescein isothiocyanate, which was obtained from ICN ImmunoBiologicals (Lisle, IL).

For localization at the electron-microscopic (EM) level, cells were fixed with glutaraldehyde (0·25–1 %) or 3 % paraformaldehyde in phosphate buffer, pH 7·6, at room temperature. Cells were washed and concentrated in 2 % gelatin for cryoultramicrotomy. After gelation, the cell pellet was cut into thin slices and subjected to increasing concentrations of sucrose up to 2·3 M (Tokuyasu, 1984). Cells were frozen and thin-sectioned at −100°C using a Reichert Ultracut E equipped with a Reichert FC 4D cyro-device. Sections were incubated in mAb, washed extensively and then incubated in goat anti-mouse IgG or IgM complexed with 10 or 15 nm gold particles (Janssen Life Science Products, Piscataway, NJ). The sections were positively stained with uranyl acetate–oxalate, and after washing were infiltrated and negatively stained with a mixture of methylcellulose and uranyl acetate (Tokuyasu, 1984). Alternatively, cells were embedded in Lowicryl K4M (Polysciences, Inc., Warrington, PA) according to the manufacturer's instructions. Lowicryl sections were incubated with mAb overnight.

For SDS–PAGE and immunoblots, all cell types were washed with inorganic salt solution (Van Wagtendonk & Soldo, 1970). After concentration by centrifugation, cells were suspended in borate (0·17 M, pH 8·2)-buffered saline containing phenylmethylsulphonyl fluoride (1 mM), iodoacetamide (9 mM), Nonidet P-40 (10 μl ml^{-1}), pepstatin A (150 μM) and aprotinin (100 Killikrein units ml^{-1}). Protein concentration was determined using the method of Lowry *et al.* (1951), with bovine serum albumin as the standard. Electrophoresis was carried out in a discontinuous vertical slab gel (10 %, Laemmli, 1970). Unless noted otherwise in the text, samples were not reduced, as most antigens become unreactive when treated with 2-mercaptoethanol. After electrophoresis, the polypeptides were transferred to nitrocellulose paper (Towbin *et al.* 1979). The nitrocellulose paper was then cut into strips, which were incubated sequentially in mAbs, rabbit anti-mouse IgG and ^{125}I-labelled protein A. Autoradiography was carried out at −80°C using Kodak intensifying screens.

Alternatively, the nitrocellulose strips were incubated with rabbit anti-mouse IgG conjugated with alkaline phosphatase (Promega Biotec, Madison, WI). The activity of this enzyme was developed, using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium as substrates, according to the manufacturer's instructions.

Results

Production of mAbs

To produce mAbs to the phagosome–lysosome system, four separate fusions were carried out during the past few years. Of the several hundred hybrid cell lines screened for each fusion, several lines were found to react with epitopes located on various parts of the trichocyst of PM$_1$. The ten such lines studied were separated into four groups on the basis of the locations of their epitopes in PM$_1$. The fact that these mAbs were raised against crude or enriched lysosomal fractions as antigens suggests that trichocyst proteins could be present within the lysosomes. To test this, differential centrifugation of a whole cell homogenate was tested for trichocyst protein lo-

cation. Most of the trichocyst proteins were found in the nuclear fraction and none was detected in either the mitochondrial or the microsomal fractions, while only one of eight mAbs showed a very weak reactivity in the lysosomal fraction (data not shown). Thus, even crude lysosomal fractions contained so few trichocyst antigens that they were, for the most part, undetectable after blotting. However, because of the powerful hybridoma technology by which a single cell responsible for one specific epitope can be isolated and grown up to a clone that then produces one specific antibody, only an extremely small amount of a relatively immunogenic antigen is required to induce antibody production. We believe that in this study mAbs were raised against very small amounts of trichocyst contaminants in the fractions and that the trichocyst proteins apparently were very immunogenic. These mAbs were very specific and did not cross-react with lysosomes or the digestive vacuoles, as shown by IFA and cryoultramicrotomy. Thus the hybridoma technique has enabled us to obtain specific antibodies without purifying each antigen.

Epitopes on the inner part (core) of the trichocyst body

After IFA, two mAbs (270D5 and 22C7F2) were found to label the central portion of the trichocysts. These labelled trichocysts looked like elongated icicles and were located in the ectoplasm as well as the endoplasm of the PM_1, seeming to fill the entire cell (Fig. 1). On the basis of these observations and those described below, these epitopes appeared to reside in the cores of the trichocyst bodies. Similar results were obtained with PM_3, *P. primaurelia*, *P. tetraurelia* and *P. caudatum*, although the staining intensity for the last species was slightly lower. In *P. calkinsi* only some brightly fluorescent lines were seen in the anterior region of the cell, but the nature of these fluorescent lines or sheets was not determined. *Tetrahymena* showed no positively labelled structures, although a diffuse fluorescence was seen. A third mAb (D8) was found to label some endoplasmic vesicles very strongly, while the cores of docked trichocysts were only lightly labelled (Fig. 5). Other species were not tested with this mAb.

At the ultrastructural level in cryosections of fixed
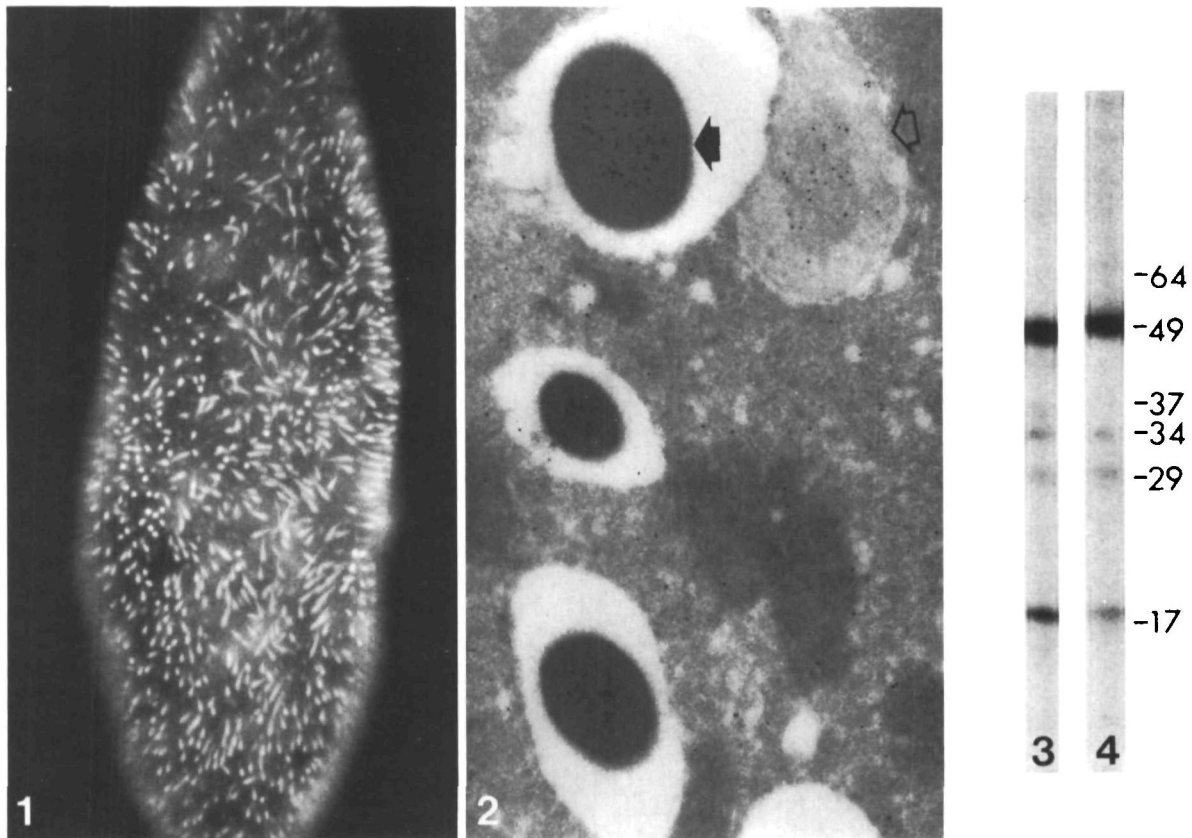
Figs 1–4. Reactivity with mAb 22C7F2.
Fig. 1. Fluorescent micrograph of PM_1 incubated with mAb 22C7F2 followed by fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG. The labelled trichocysts resembled icicles. ×550.
Fig. 2. Localization of the epitopes for mAb 22C7F2 in frozen thin sections of PM_1 that was fixed in 1 % glutaraldehyde for 20 min. Both the cores of the mature (filled arrowhead) and immature (open arrowhead) trichocyst bodies were labelled with immunogold reagents. The empty space between the body and the unpreserved trichocyst membrane was an artefact related to the procedures of frozen thin sections. ×20 000.
Figs 3–4. Western immunoblots of two different homogenates of PM_1 probed with mAb 22C7F2. Both homogenates showed weak reactions with the 29–40 ($\times 10^3$) M_r protein family, but the intensity of reactivity with the 17×10^3 band was much reduced in the homogenate shown in Fig. 4.

PM₁, these three mAbs specifically labelled the core of the trichocyst body, which in all cases was surrounded by an unreactive shell of $\approx 0.16 \mu m$ in width (Figs 2, 6, 7). For mAb 22C7F2, the only one studied extensively, the label was located on condensed (filled arrow, Fig. 2) as well as partially condensed (open arrow, Fig. 2) trichocyst bodies (refer to Hausmann et al. 1988, for additional studies using this mAb). From comparison of their reactivities in cryosections, mAb 270D5 appeared to be weaker than mAb 22C7F2, as fewer gold particles were seen on trichocyst cores (data not shown). The epitopes for mAb 22C7F2 were fairly stable and remained reactive even after fixation with 1% glutaraldehyde, although increasing concentrations of glutaraldehyde resulted in decreasing label intensities. Background labelling was very low with mAbs 22C7F2 and 270D5, but relatively high with mAb D8 (Fig. 7). No cryoultramicrotomy was carried out with the other cell species.

For electrophoresis, whole cell homogenates of the six species of cell were obtained after washing cells in an inorganic salt solution. After SDS–PAGE under non-

reducing conditions and electroblotting, the epitopes for these three mAbs were found to be located within three families of trichocyst proteins. The mAb 270D5 reacted strongly with proteins of the 34 and 29 but weakly with the $37 (\times 10^3) M_r$ bands (Fig. 9A). Treatment with 2-mercaptoethanol prior to electrophoresis completely abolished the antigenicity for this mAb. The mAb 22C7F2, on the other hand, reacted with all three families of antigens, although the reactivity of this mAb for the two families of lower molecular weight antigens varied from one homogenate to another (Figs 3, 4, 9G). A faint reaction with the $29–40 (\times 10^3) M_r$ family in two different homogenates (Figs 3, 4) and a strong reaction with the $29 \times 10^3 M_r$ band in another (Fig. 9G) homogenate were observed. Also the intensity of the $17 \times 10^3 M_r$ band ranged from strong (Fig. 3) to weak (Fig. 4) to very faint (Fig. 9G). The reason for these variations in reactivity are not at present known, but will be explored later when the relationships among these three families of trichocyst antigens are studied. When rabbit anti-mouse IgG conjugated with alkaline phosphatase was used as the second
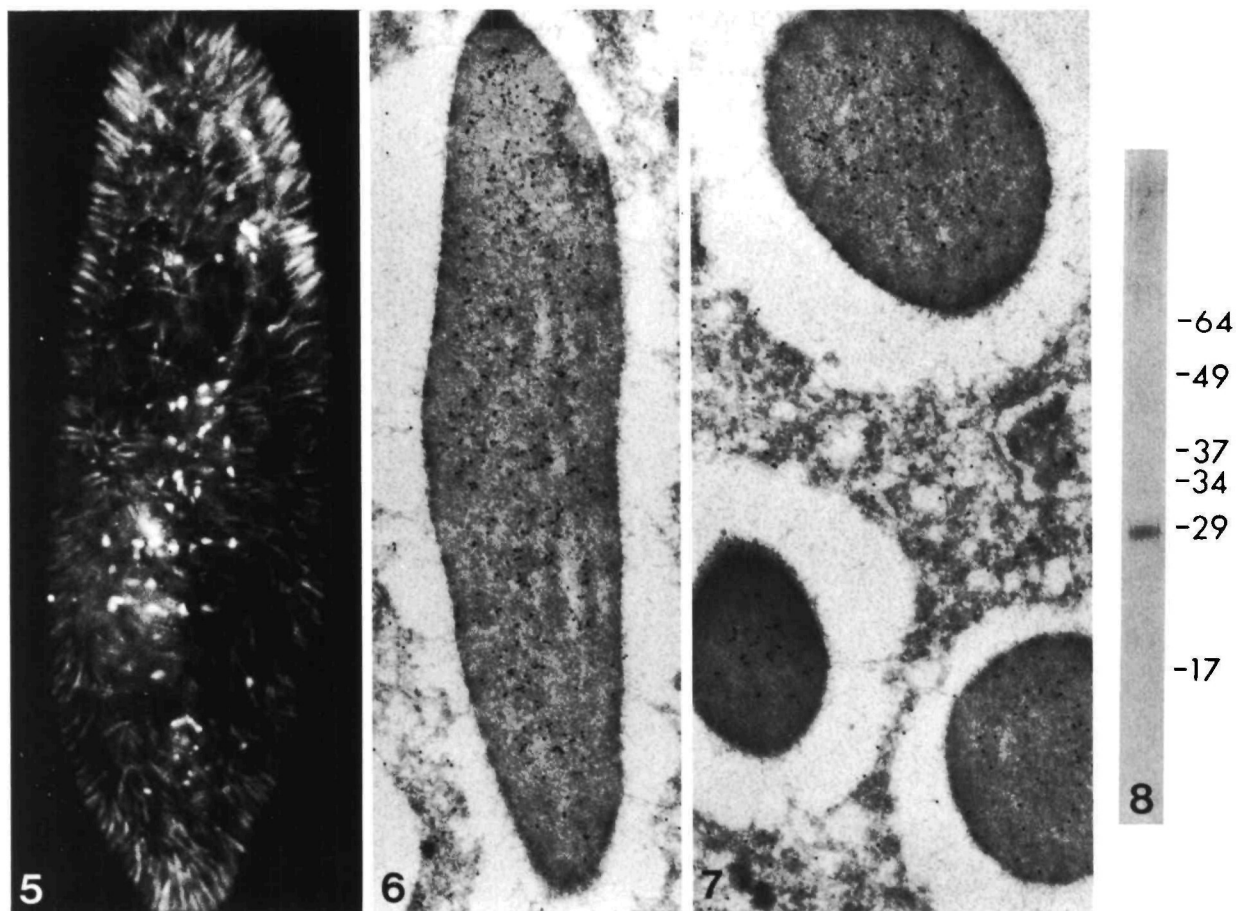
Figs 5–8. Reactivity with mAb D8.
Fig. 5. Fluorescent micrograph of PM₁ incubated with mAb D8. Many vesicles were brightly labelled, while the cores of mature trichocysts were moderately labelled. ×550.
Figs 6–7. Localization of epitopes for mAb D8 in frozen thin sections of PM₁ that was fixed in 0.25% glutaraldehyde for 20 min. Label was concentrated in the core of the resting trichocysts in longitudinal (Fig. 6) and cross-sections (Fig. 7). The unreactive body shell measured about $0.16 \mu m$. Background label was higher than that shown in Fig. 2. ×22 000.
Fig. 8. Immunoblot of PM₁ homogenate probed with mAb D8. The epitope for this mAb was located on the $29 \times 10^3 M_r$ band.

antibody, similar results were obtained with mAbs 270D5 and 22C7F2, although with the latter mAb a doublet at 47 and 49 $(\times 10^3) M_r$ instead of only one protein band was observed (not shown). The epitopes for mAb D8 were located on a $29 \times 10^3 M_r$ band in PM_1 (Fig. 8); the D8 antigenic sites in the other cell species were not studied.

While trichocyst cores in all paramecia except *P. calkinsi* were labelled with mAbs 270D5 and 22C7F2 using IFA, the M_r of these antigens varied slightly (for comparison, experiments with five paramecia and with *Tetrahymena* homogenates were carried out under identical conditions). The typical reactivity of PM_3 cells with each of these two mAbs (Fig. 9B,H) was similar to that for PM_1 (Fig. 9A,G). However, with the PM_3 homogenate used to produce Fig. 9H, the $17 \times 10^3 M_r$ band was absent when incubated with mAb 22C7F2. In *P. primaurelia* mAb 270D5 reacted strongly with the wide 34–31 $(\times 10^3) M_r$ band (Fig. 9C), while all three families of antigens reacted with mAb 22C7F2 (Fig. 9I). In *P. tetraurelia* mAb 270D5 reacted with a doublet of 36 and 34 $(\times 10^3 M_r$, Fig. 9D) and the two higher M_r families were weakly reactive with mAb 22C7F2 (Fig. 9J). In *P. caudatum* (Fig. 9E,K) and in *Tetrahymena* (Fig. 9F,L), both mAbs reacted with the family of proteins of 29–40 and 64 $(\times 10^3) M_r$, respectively. With *P. calkinsi*, mAb 22C7F2 reacted with both a 37 and a broad 28–31 $(\times 10^3) M_r$ band, while only the former band was reactive with mAb 270D5 (data not shown).

Epitopes located on the beaded or meshlike sheath of the trichocyst body

Four mAbs (131C1E8, A1-3, A16-2 and D7) were found to react with the outer portion of the trichocyst body in PM_1 using IFA. The label was seen as circular profiles in end-on views with the inner core unlabelled (Fig. 10). The overall diameter of the labelled trichocyst profiles was bigger than those seen in Fig. 1, whose labelled core appeared to be roughly the right size to be accommodated in the unlabelled space inside these labelled shells. Similar results were obtained with the different paramecia tested. Trichocysts in *P. calkinsi* were labelled very brightly with mAb A16-2, but not with the other three mAbs, while *Tetrahymena* showed no specific label with these four mAbs.

Of the four mAbs in this group, mAb 131C1E8 gave the best results and was reactive after 0·25 % glutaraldehyde fixation. In Lowicryl K4M-embedded sections, preliminary results showed that this mAb labelled specifically only the outer edge, previously called the beaded (Bannister, 1972) or the meshlike (Anderer & Hausmann, 1977) sheath of the docked trichocysts (Fig. 11). It is not known whether the outer edges of partially condensed trichocysts and primordial vesicles are labelled. Little label was seen in other areas on sectioned trichocysts and background label was relatively low.

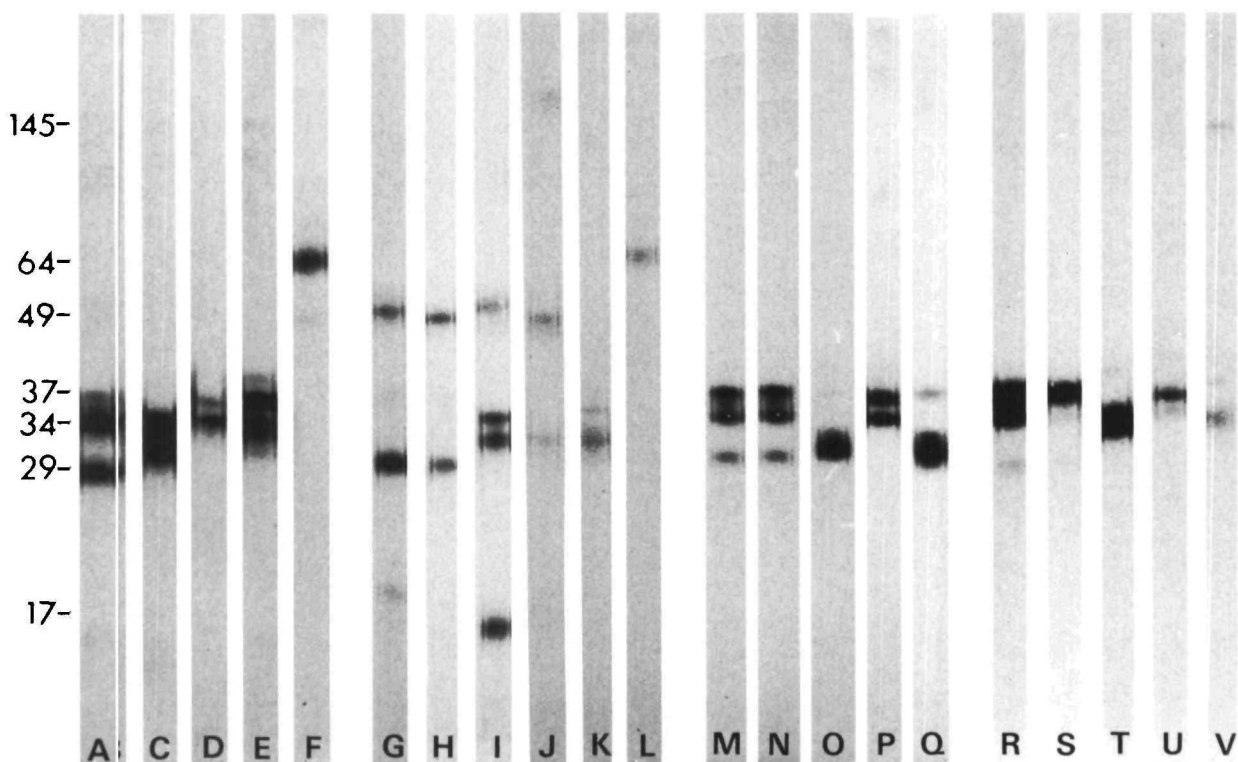After SDS–PAGE under non-reducing conditions, all four mAbs reacted very strongly with a trio of well-

Fig. 9. SDS–PAGE and Western blotting were carried out under identical condition. A–F. Immunoblots of homogenates from PM_1 (A), PM_3 (B), *P. primaurelia* (C), *P. tetraurelia* (D), *P. caudatum* (E) and *Tetrahymena* (F) probed with mAb 270D5.
G–L. Immunoblots of the same six homogenates in the same order as in A–F were probed with mAb 22C7F2.
M–Q. Immunoblots of five paramecia homogenates in the same order as in A–E were probed with mAb 131C1E8.
R–V. Immunoblots of five paramecia homogenates in the same order as in A–E were probed with mAb 135B9E7.

−64
−49
−37
−34
−29

−17

12
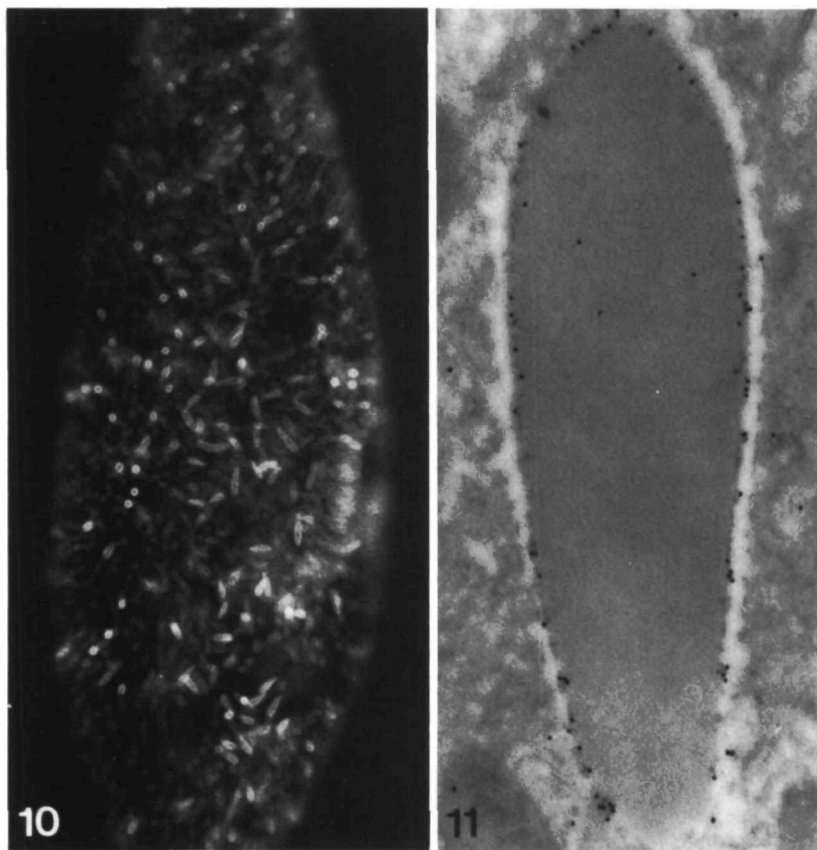
Figs 10–12. Reactivity with mAb 131C1E8.
Fig. 10. Fluorescent micrograph of PM₁ incubated with mAb 131C1E8, which serves as a representative of the other three mAbs (A1-12, A16-2 and D7), which have identical specificity. ×550.
Fig. 11. Localization of epitopes of mAb 131C1E8 in Lowicryl K4M-embedded PM₁ that was fixed in 0·25 % glutaraldehyde in phosphate buffer containing 2·5 % sucrose for 30 min. Label was restricted to the very outer edge of the trichocyst body. The section was not post-stained with uranyl acetate or lead citrate. ×32 000.
Fig. 12. Immunoblot of PM₁ homogenate probed with mAb 131C1E8 showing the typical strong reactivity with three well-defined bands of 37, 34 and 29 $(\times 10^3)\,M_\mathrm{r}$.

defined bands of 37, 34 and $29(\times10^3)\,M_\mathrm{r}$ in PM₁ (Figs 9M, 12) and PM₃ (Fig. 9N). In some experiments additional moderately labelled bands could also be seen between these three prominent bands (Fig. 9M,N). *P. primaurelia* (Fig. 9O), *P. caudatum* (Fig. 9Q) and *P. calkinsi* (data not shown) had one broad band between 28 to 31 $(\times10^3)$, while *P. tetraurelia* had two distinct bands of 36 and 34$(\times10^3)$ (Fig. 9P). Again, no reactivity was observed with *Tetrahymena*.

Epitopes on the cortex of the trichocyst body

Using IFA, mAb 135B9E7 was found to react with epitopes located on a subpopulation of trichocysts; the most reactive of these trichocysts did not appear to be the docked trichocysts, because they were concentrated in the endoplasm (Fig. 13). In end-on views, the cortex but not the core of the trichocyst body fluoresced, so that rings could be seen. However, the fluorescence outlining the trichocysts appeared to be either more intense or thicker than that in Fig. 10, suggesting some differences in the disposition of these epitopes compared with the peripherally disposed group described above. Similar results were observed for PM₃ and the two species of the *aurelia* group, while a diffuse staining characterized *P. caudatum*. *Tetrahymena* was unlabelled.

Using cryoultramicrotomy followed by immunostaining, preliminary results confirmed that the epitopes for this mAb were located on the body cortex of the trichocysts (Figs 14, 15). The thickness of the labelled cortex was about 0·16 μm. Interestingly, in cryosections all trichocysts, docked as well as endoplasmic, appeared

to be equally labelled. Background label in this experiment was relatively high.

After SDS–PAGE, this mAb was shown to label two bands of 37 and 34 strongly and 29 $(\times10^3)\,M_\mathrm{r}$ weakly in PM₁ (Figs 9R, 16). These bands appeared to be similar in their molecular weights to the trio of bands that were located on the beaded sheath as well as the core of the mature trichocysts (see Figs 9, 12). With PM₃ only the $37\times10^3\,M_\mathrm{r}$ band was strongly reactive (Fig. 9S). With the other three paramecia only one of the trio of polypeptide bands was significantly labelled (Fig. 9T–V) and with *P. calkinsi* only a 28–31 $(\times10^3)\,M_\mathrm{r}$ broad band was reactive (data not shown).

Epitopes located on trichocyst tips

After IFA, two mAbs (59E4F4 and 282C11) gave a strong and rather distinct surface pattern of punctate fluorescence that appeared to correspond to the sites of trichocyst tips (Fig. 17). The tips of all other paramecia, except for *P. calkinsi*, were labelled with these mAbs. *Tetrahymena* was unlabelled. The locations of the epitopes were verified in frozen thin sections where the epitopes were found to be located on the sheath covering the paracrystalline tip as seen in longitudinal (Fig. 18) as well as cross-sections (Fig. 19). Since these epitopes in PM₁ were not reactive after SDS–PAGE in either reducing or non-reducing conditions, their presence in the other cell types was not studied.

Reactivity with discharged trichocyst contents

To show that the mAbs were directed to trichocyst
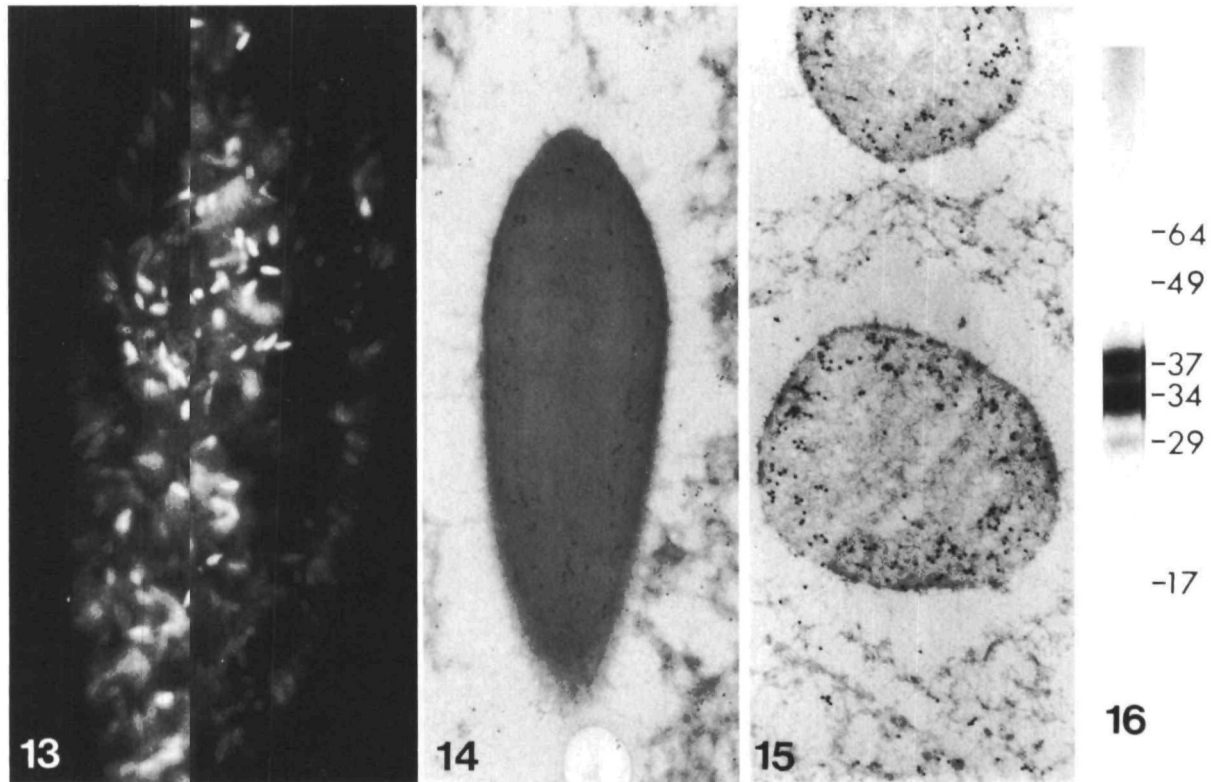
Figs 13–16. Reactivity with mAb 135B9E7.
Fig. 13. Fluorescent micrograph of PM₁ incubated with mAb 135B9E7. Compared with Fig. 10, fewer trichocysts were labelled with this mAb, but the labelling intensity was higher and the circular profiles appeared thicker. ×550.
Figs 14–15. Localization of the epitopes for mAb 135B9E7 in frozen thin sections of PM₁ that were fixed in 0·5 % glutaraldehyde for 45 min (Fig. 14) and 0·25 % glutaraldehyde for 20 min (Fig. 15). Label was located specifically on the cortex (about 0·16 μm in thickness) of the trichocysts in longitudinal (Fig. 14) and cross (Fig. 15)-sections. ×23 760.
**Fig. 16.** Immunoblot of PM₁ homogenate probed with mAb 135B9E7 showing a typical strong reactivity with two of the trio of bands and weak reactivity with the $29 \times 10^3 M_r$ band.

proteins, two preparations of discharged trichocysts were made. For the first preparation, which was obtained from cells subjected to a cold shock and was used without further purification, the middle family $(29–40\,(\times 10^3)\,M_r$ as compared with the molecular weight markers shown in Fig. 20A), two polypeptides of the third family (18 and $20\,(\times 10^3)\,M_r)$ and numerous faint bands typically found in whole cell homogenates were seen (Fig. 20B). The second preparation, which was purified using a raffinose cushion as described by Adoutte *et al.* (1984), was cleaner. The protein bands for the middle family were seen, but the 18 and $20\,(\times 10^3)\,M_r$ bands were almost absent (Fig. 20D). These results agreed with those obtained by Peterson *et al.* (1987), who reported that under non-reducing conditions the discharged tricho-cysts consisted of bands between 30 and $35\,(\times 10^3)\,M_r$. When the blots of these two preparations were probed with mAb 131C1E8, a labelling pattern identical to that obtained with whole cell homogenates (Fig. 20C,E) was observed. These results leave little doubt that the mAbs were directed to the trichocyst proteins.

Discussion

Using the hybridoma techniques to produce membrane

markers for the phagosome–lysosome system in PM₁, we have serendipitously obtained 10 mAbs to various epi-topes on trichocysts. However, before they can be used to study trichocyst formation or secretion, it is necessary to characterize them completely using morphological and biochemical techniques. On the basis of the IFA and cryoultramicrotomy, four mAbs have been shown to be directed to antigens on the beaded (Bannister, 1972) or meshlike (Anderer & Hausmann, 1977) body sheath, one to the cortex, three to the core and two to the sheath around the tip of the trichocysts. Using SDS–PAGE and electroblotting, we showed that eight of the ten mAbs reacted with selected bands of the three families of closely migrating polypeptides of 40–55, 29–40 and $15–20\,(\times 10^3)$ M_r, respectively (Steers *et al.* 1969; Adoutte *et al.* 1984). All three families appear to be represented on the core, while only the $29–40\,(\times 10^3)\,M_r$ family is seen on the beaded sheath and the cortex. These results provide some antigenic support for the reported morphological differences seen in the beaded body sheath and the body cortex, which in thin sections of the undischarged trichocysts is more electron-dense than the body core (see figs 4, 10 of Anderer & Hausmann, 1977; fig. 11 of Bannister, 1972). Whether these morphological differences and the different locations of the epitopes for
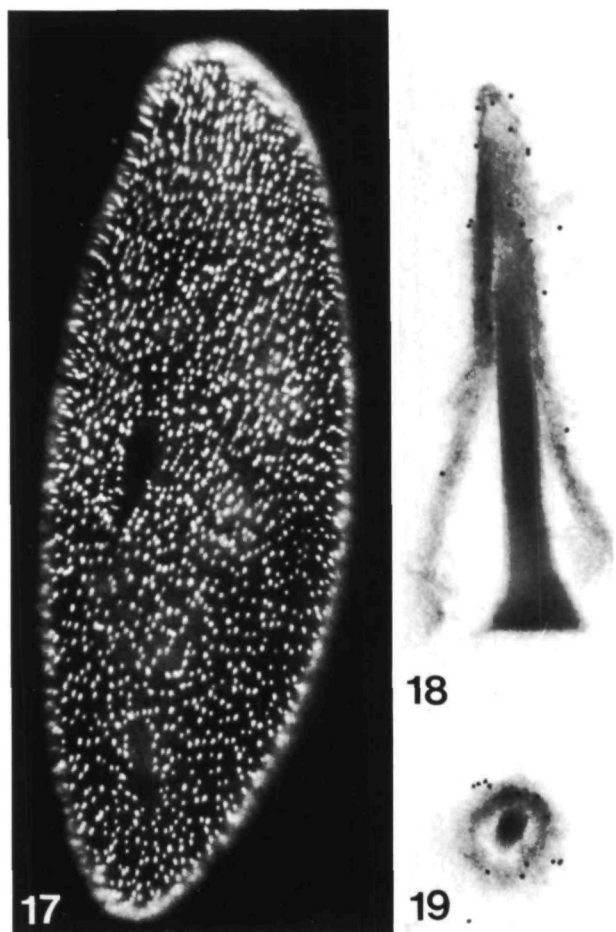
Figs 17-19. Reactivity with mAb 59E4F4.
Fig. 17. Fluorescent micrograph of PM_1 incubated with mAb 59E4F4, showing a punctate pattern at the cell surface. ×550.
Figs 18-19. Localization of the epitopes for mAb 59E4F4 in frozen thin sections of PM_1 that was fixed in 3 % paraformaldehyde for 30 min. Label was located on the trichocyst sheath covering the paracrystalline tip as seen in longitudinal (Fig. 18) and cross (Fig. 19)-sections. ×40 000.
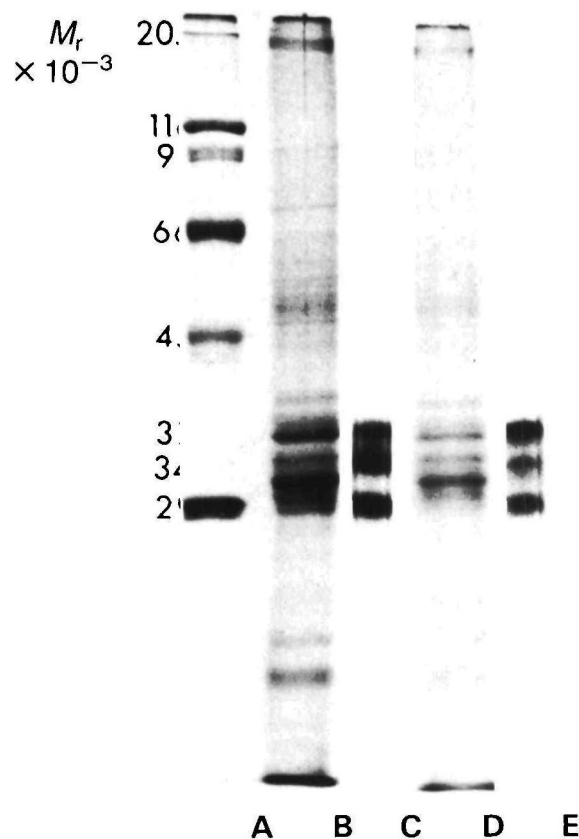

Fig. 20. SDS-PAGE pattern of discharged trichocysts under non-reducing conditions and their reactivity with mAb 131C1E8. A. Molecular weight markers were, from top to bottom: myosin, β-galactosidase, phosphorylase, bovine albumin, egg albumen and carbonic anhydrase. B. Profile of a crude preparation of discharged trichocysts. C. Immunoblot of the crude discharged trichocysts shown in B. D. Profile of a purified preparation of discharged trichocysts.
E. Immunoblot of the purified discharged trichocysts shown in D.

the various mAbs are related to differences in the structural arrangements or in chemical composition of the proteins on the beaded sheath, cortex and core is not known, although a recent study has reported that the central core proteins are more heat-labile than those on the beaded sheath (or possibly the cortex) of the extruded trichocyst body (Peterson et al. 1987).

The results obtained in this study shed new light on an earlier study by Adoutte et al. (1984), who suggested that the 47 and the 15-20 $(\times 10^3) M_r$ protein families are precursor and product, respectively, and that during maturation cleavage of the former gives rise to the latter. We interpret our results to suggest that, at least in PM_1, both the 47 and the 29-40 $(\times 10^3) M_r$ protein families are final products because these are present in docked trichocysts. What, then, do the lowest molecular weight family, the 15-20 $(\times 10^3) M_r$ polypeptides, represent? If they are the only final polypeptide products, then the reactivity of this M_r family with mAb 22C7F2 should be

more intense and less variable, as there are thousands of docked trichocysts in *Paramecium*. Furthermore, under non-reducing conditions, this family of proteins is absent in the SDS-PAGE profile of the purified discharged trichocysts (Fig. 20D) and in those of Peterson et al. (1987). To resolve these questions, further experimentation is being carried out.

In summary, this is the first report of a panel of mAbs raised against trichocysts. These mAbs have been shown to be specific for selected polypeptides of the trichocysts and they label this organelle at different sites as seen in IFA and at the ultrastructural level. These mAbs also cross-react with all species of *Paramecium* studied and give characteristic blots that may be used to identify these species. Owing to their specificities, these mAbs can now be used to study trichocyst protein development, the relationships among trichocyst protein families, and the changes occurring in these proteins during condensation and decondensation during trichocyst extrusion.

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