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Inhibition of development in *Myxococcus xanthus* by monoclonal antibody 1604

(submerged culture development/contact-mediated cell interactions/cell surface antigen/affinity purification)

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ABSTRACT **Monoclonal antibody (mAb) 1604 is directed against a cell surface antigen of *Myxococcus xanthus*. Purified antibody 1604 inhibited development of *M. xanthus* under conditions of submerged culture procedure otherwise leading to fruiting body formation. Intact molecules of mAb 1604, as well as its Fab fragments, inhibited developmental aggregation, autolysis, fruiting body formation, and sporulation. The addition of relatively small amounts of antibody every 4 hr was much more effective than a single large dose given at the onset of development. The inhibitory action of mAb 1604 on development was reversible after prolonged incubation of the antibody with cells; this was probably due to proteolytic degradation of the antibody. The effect of mAb 1604 on submerged bacterial development was neutralized by affinity-purified 1604 cell surface antigen. Another antibody, mAb 2788, directed against an *M. xanthus* cell surface antigen, did not block development. These data suggest that 1604 cell surface antigen is involved in contact-mediated cell interactions in *M. xanthus*.**

Appropriate environmental conditions initiate a set of developmental events in *Myxococcus xanthus* that lead to aggregation, developmental autolysis, and formation of fruiting bodies and myxospores (1–4). During these events, a number of cell–cell interactions occur that seem to require the exchange of cell-to-cell signals. A few examples of these cellular interactions are developmental aggregation (4), formation of fruiting bodies (2), developmental autolysis (3), rippling (5), feeding (6), cell density determination (7), social motility (8, 9), and extracellular complementation (10–12). These signals may be exchanged via diffusible extracellular compounds (7) or by direct cell-to-cell contact (9, 12). While social motility (9) and one aspect of extracellular complementation (12) seem to be mediated by cell-contact interactions, in neither case has this been unequivocally demonstrated, nor has the mechanism been defined.

It is possible to screen monoclonal antibodies (mAb) that have been raised randomly against surface antigens of cells for the ability to block developmental or interactional events. The ability of mAbs to block or modify such an event is thus preliminary evidence that the corresponding antigen is involved in the process. This strategy has been successfully used to study cell–cell adhesion in *Polysphondylium pallidum* (13, 14) and vertebrate brain cells (15), aggregation in *Dictyostelium discoideum* (16–18), and cell–substrate adhesion in chicken neural cells (19). This approach has not been used in the study of prokaryotic cell–cell interactions.

In this report we describe a mAb (1604), directed against a cell surface antigen of *M. xanthus*, that inhibited aggregation, developmental autolysis, sporulation, and fruiting body formation. These effects were neutralized by pretreatment of the mAb with an affinity-purified preparation of corresponding cell surface antigen.

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MATERIALS AND METHODS

Organism and Growth Conditions. *M. xanthus* DK1622 (8), designated as MD207 in our laboratory, was used throughout this study. The procedure for growth and submerged development has been described previously (20). Briefly, cells in CTT medium (9) were grown in tissue culture plates for 24 hr during which time they became attached to the plastic surface. The cells were rinsed with distilled water, and Mops/salts buffer solution was added to initiate development. The Mops/salts buffer contained 10 mM Mops (4-morpholine propanesulfonic acid; Sigma), 2 mM $CaCl_2$, 4 mM $MgSO_4$, and 50 mM NaCl, pH 7.2. Growth, development, and the assay of the effect of the mAbs on development were done in 96-well tissue culture plates (Costar, Cambridge, MA).

Determination of Numbers of Vegetative Cells and Myxospores. Vegetative cells were fixed in 2.5% glutaraldehyde for 19 hr at 4°C, released from the plastic surface of the tissue culture plates by sonic oscillation for 5 sec at 50 W (Sonic Cell Disruptor, Heat Systems/Ultrasonics, Plainview, NY), and counted in a Petroff–Hausser counter. Determination of myxospore numbers was done as follows: cells and fruiting bodies were subjected to sonic oscillation for 55 sec at 50 W and then incubated for 2 hr at 51°C. This procedure killed vegetative cells and dispersed myxospores from the fruiting bodies (12). This suspension was plated on CTT agar (9).

Production, Purification, and Fragmentation of mAbs. The procedure for the production of mAbs 1604 and 2788 has been described (20). mAbs were produced in large quantity by generating ascites fluid in BALB/cWat mice (University of Minnesota, Minneapolis, MN). The mAbs were purified from ascites fluid using a protein A–Sepharose CL-4B affinity chromatography column (Pharmacia). Purified mAbs were dialyzed against a buffer solution containing 10 mM Mops and 50 mM NaCl, pH 7.2. The fragmentation of mAb 1604 into Fab fragments was done by the method of Oi and Herzenberg (21). The Fab fragments were purified using protein A–Sepharose CL-4B affinity chromatography.

Preparation of Affinity-Purified Cell Surface Antigen. Approximately 2×10^9 vegetative cells of *M. xanthus* were lysed by osmotic shock in the presence of the protease inhibitor, phenylmethylsulfonyl fluoride (Sigma), treated with DNase I (Sigma), solubilized in 100 mM $NaHCO_3$/100 mM NaCl/0.1% Zwittergent 3-14 (*N*-tetradecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate; Calbiochem–Behring), pH 7.5, and loaded onto a mAb 1604-CNBr-activated Sepharose 4B column prepared by the method of Pharmacia (22). The column was washed with bicarbonate buffer, and the antigen was eluted with 50 mM diethylamine (Sigma)/100 mM NaCl/10 mM Tris/0.1% Zwittergent 3-14, pH 11.5 (23). The

Abbreviation: mAb, monoclonal antibody.

antigen solution was concentrated by dialysis against polyethylene glycol. It was then dialyzed against bicarbonate buffer with 0.05% Zwittergent 3-14. Protein concentration was determined by a Lowry assay modified to tolerate detergent (24).

Assay for Biological Activity of mAbs and Cell Surface Antigen. mAbs 1604 and 2788 were assayed for the ability to interfere with normal submerged development by periodically adding 6 μg of purified mAb to 100 μl of buffer in each well of 96-well tissue culture plates; addition of antibody began at 0 hr of development and continued every 4 hr for 32 hr. Light micrographs were taken of the contents in the wells using a Zeiss inverted microscope of 40× total magnification. The assay of mAb 1604 Fab fragments was similar to the assay of intact 1604 antibody; the only difference was that 20 μg of Fab fragments were added periodically instead of 6 μg of intact antibody. Neutralization of the development-inhibiting effect of mAb 1604 by the corresponding cell surface antigen was done by mixing together 220 ng of cell surface antigen preparation, from which the detergent had been removed by Amberlite XAD-2 (Sigma), and 30 μg of mAb 1604 in 100 μl, incubating for 1 hr at 20°C, and then adding the total amount of antigen/antibody mixture to the cells only at 0 hr of development.

RESULTS

Kinetics of Autolysis and Sporulation During Submerged Development. The procedure for submerged culture development was described previously by Kuner and Kaiser (25) and modified by Gill and Dworkin (20). This modified procedure significantly accelerated the sequence of developmental events (Fig. 1). The relationship of growth, cell lysis, and sporulation is seen in Fig. 2. During vegetative growth in CTT medium, the number of cells increased from 1×10^7 cells per ml to 2×10^8 cells per ml. After 8 hr in the buffer that induces development, cell lysis began and continued until ≈90% of the cell population had lysed at 72 hr. Sporulation began between 28 and 32 hr and was nearly completed by 48 hr, at which time $\approx 4 \times 10^6$ spores per ml were present.

Effect of mAbs 1604 and 2788 on Submerged Development. The process of development was almost completely inhibited

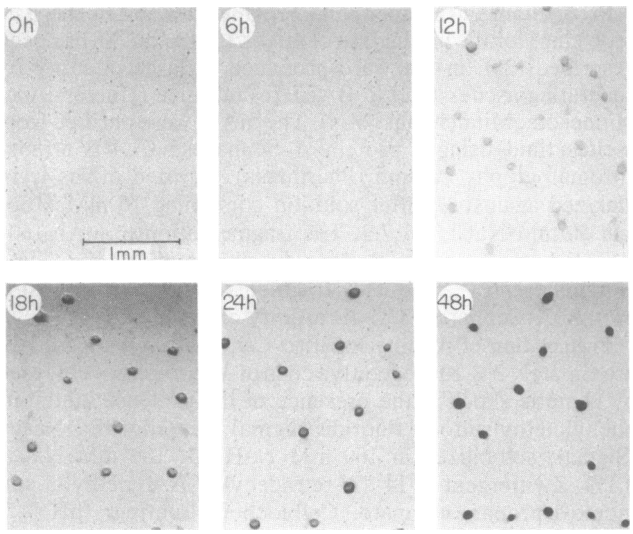

FIG. 1. Gross morphological changes during development that occurs under submerged bacterial culture. Photographs were taken of *M. xanthus* under development-inducing conditions in 96-well tissue culture plates. Stages of development are as follows: 0 hr, vegetative cell mat; 6 hr, mat of preaggregation cells; 12 hr, early aggregates; 18 hr, mature aggregates; 24 hr, immature fruiting bodies; and 48 hr, mature fruiting bodies.

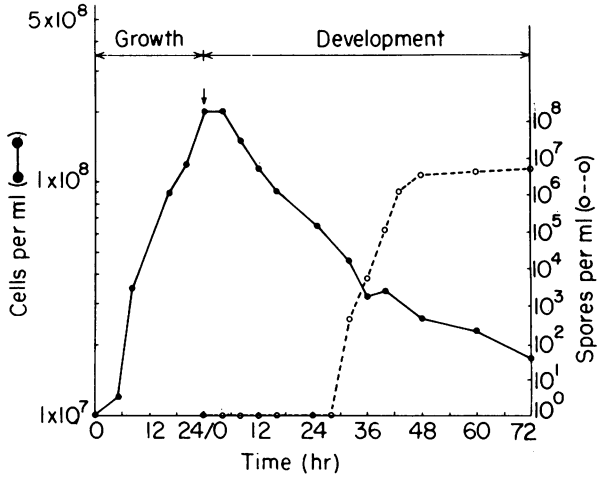

FIG. 2. Growth, developmental autolysis, and sporulation. Cells were grown in CTT medium; determination of the number of cells and myxospores is described. Cells were placed under conditions that induce development at the time indicated by the arrow.

by mAb 1604 (Fig. 3*A*). To obtain this degree of inhibition, 6 μg of mAb 1604 was added every 4 hr to 100 μl of cells under development-inducing conditions in wells of 96-well tissue culture plates. While no evidence of aggregation was observed at 12 hr, rippling (rhythmic oscillations) (5) occurred. This is seen as the somewhat parallel lines in Fig. 3*A* at 12, 24, and 32 hr. Rippling continued for the duration of the antibody-addition period. Neither aggregates nor fruiting bodies formed during the addition period (32 hr) of mAb 1604. Furthermore, cell lysis and sporulation did not occur in the presence of mAb 1604 (Table 1). By 88 hr after the final addition of antibody, sufficient proteolytic degradation of the antibody had occurred (data not shown) to allow cell lysis and sporulation.

The effect of mAb 1604 Fab fragments on submerged development was similar to that of intact 1604 antibody (Table 1). The formation of aggregates and fruiting bodies was nearly completely inhibited (data not shown), and cell lysis and sporulation were significantly suppressed (Table 1).

Identical experiments using mAb 2788 showed no effect on development. The timing of aggregation and fruiting body formation was identical to the buffer control (Figs. 1 and 3*B*). Cell lysis and sporulation were also not affected (Table 1). ImmunoGold labeling with mAb 2788 indicated that, like mAb 1604, the corresponding antigen was located on the cell surface (data not shown).

Recovery from mAb-Induced Inhibition of Development. *M. xanthus* secretes extracellular proteases (26, 27). mAb 1604 was degraded by proteolytic activity under developmental conditions (data not shown). Thus, frequent periodic additions of small amounts of antibody were more effective than a single large addition at the beginning of development. The inhibitory effect was completely reversible if the periodic antibody additions to arrested cells were discontinued (Fig. 4). The inhibitory effect of 9 individual additions of mAb 1604 every 4 hr (6 μg per addition) for 32 hr was first seen to be reversed by ≈18 hr following the final antibody addition. Once aggregation began (18 hr), development proceeded at an accelerated rate compared with that of the buffer control, and within only 3 hr more, mature aggregates/immature fruiting bodies had formed. Cell lysis and sporulation returned to levels seen in the mAb 2788 control and the buffer control (Table 1). Recovery from 1604 Fab fragment-induced inhibition also occurred but was much slower than recovery seen with intact 1604 antibody (Table 1).

Neutralization of mAb-Induced Inhibition of Development by Cell Surface Antigen. The inhibitory effect on development

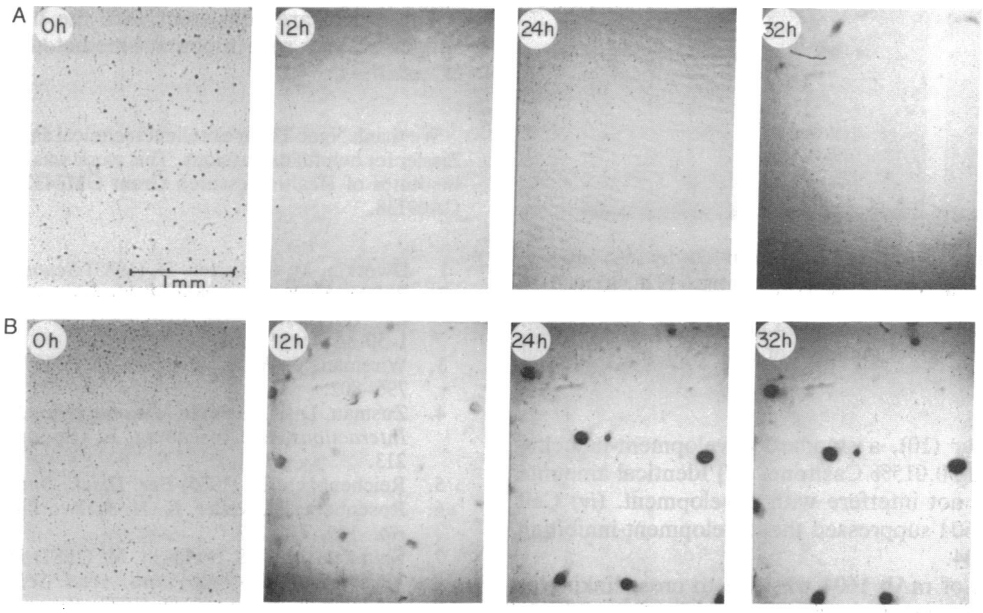

FIG. 3. Effect of mAbs 1604 and 2788 on submerged culture development. Six micrograms of purified mAbs were added every 4 hr for 32 hr to microtiter wells containing 100 μl of cells. (*A*) mAb 1604; (*B*) mAb 2788. Note the occurrence of rippling (rhythmic oscillations) in *A*.

by mAb 1604 was neutralized by incubating mAb 1604 with its corresponding antigen before adding the antibody–antigen mixture to cells (Fig. 5*C*). This mixture was added in a single dose when the cells were first placed under developmental conditions. For reasons that were not clear, the shape of aggregates and fruiting bodies was distorted, indicating that mAb 1604 antigen did not completely neutralize the effect of mAb 1604 on development. As shown in Fig. 5*B*, this amount of affinity-purified cell surface antigen (220 μg per well), when added alone, had no effect on fruiting body formation, although amounts greater than this interfered with development.

DISCUSSION

We have generated 31 mAbs against antigens located on the cell surface of *M. xanthus*. These mAbs were directed either against shared antigens found on both vegetative and developing cells or against antigens found only on developing cells (20, 28). A number of the purified antibodies have the ability to interfere with the developmental process of *M. xanthus in vivo*. mAb 1604, which reacts with an antigen present on both vegetative and developing cells, when added periodically in small amounts to cells placed under submerged fruiting conditions, inhibited aggregation, fruiting body formation, cell lysis, and sporulation.

The frequent addition of mAb 1604 to developing cells was found to be more effective at inhibiting development than a single exposure to antibody. *M. xanthus* secretes extracellular proteases (26, 27), and we have found that proteases were also secreted into the developmental buffer during development. Using $NaDodSO_4$/PAGE analysis, we have determined that mAb 1604 was degraded by *M. xanthus* proteases (data not shown). Addition of antibody every 4 hr, thus, provided a constant supply of active antibody to interfere with development (Fig. 3*A*). On the other hand, a single addition of antibody (Fig. 5*A*), although capable of inhibiting development, usually did not persist as long as small amounts added frequently.

Myxobacteria hydrolyze extracellular macromolecules and utilize the products for growth (6). There was a possibility that the inhibitory effect of mAb 1604 was not antibody–antigen specific but instead was a nonspecific nutritional effect. However, we have ruled out any nutritional effects based on the following: (*i*) Inhibition of development was observed after the addition of only 9 μg of antibody (0.009%). This amount was far less than the minimum amount of hydrolyzed casein (Casitone, Difco) necessary to sustain growth in *M. xanthus* strain FB (6). (*ii*) Development readily

Table 1. Effect of mAbs on cell lysis and sporulation

	Lysis, %		Myxospores, no. per ml	
	16 hr*	88 hr	16 hr	88 hr
Buffer control	83	91	3.4×10^6	2.6×10^7
1604 IgG	0	88	2.8×10^2	4.8×10^7
1604 Fab	0	20	3.4×10^2	2.2×10^4
2788 IgG	77	89	4.2×10^5	2.7×10^7

At 0 hr, there were 2.0×10^8 cells/ml.
*hr in this row represents time after the final addition of antibody. Intact IgG antibodies were added at 6 μg/well; Fab fragments were added at 20 μg/well.

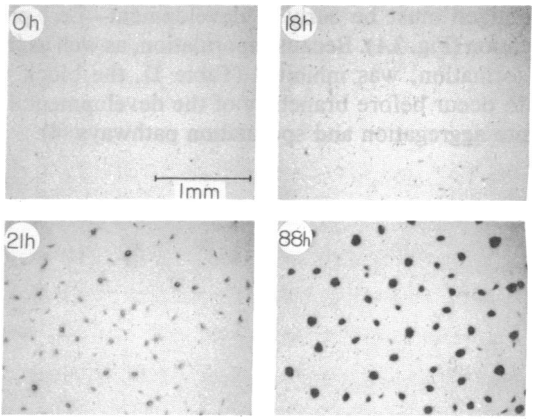

FIG. 4. Recovery from developmental inhibition induced by mAb 1604. mAb 1604 was added to cells for 9 separate additions (as for Fig. 3). Timing of recovery began at the point of the last antibody addition. 0 hr, mat of vegetative cells; 18 hr, very early aggregation; 21 hr, aggregation; and 88 hr, fruiting bodies.

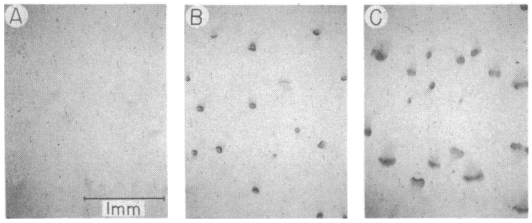

FIG. 5. Neutralization of the effect of mAb 1604 by 1604 antigen. mAb 1604 (30 μg) was incubated with 1604 antigen (220 ng) for 1 hr and then added to cells only once at the beginning of developmental induction. Photographs were taken at 24 hr. (*A*) mAb 1604 (30 μg); (*B*) 1604 antigen (220 ng); (*C*) mixture of incubated mAb 1604 and 1604 antigen.

occurs on CF agar (10), a standard development-inducing medium containing 0.015% Casitone. (*iii*) Identical amounts of mAb 2788 did not interfere with development. (*iv*) Cell surface antigen 1604 suppressed the development-inhibiting effect of mAb 1604.

That the effect of mAb 1604 was due to cross-linking or clumping of cells via antibody–cell matrices was possible. In that case, development would have been inhibited by immobilizing the cells—preventing the freedom of movement that may be necessary for developmental processes. Effects due to cross-linking were eliminated by using mAb 1604 Fab fragments. Although the concentrations necessary to inhibit development were greater (20 μg/well), the 1604 Fab fragments yielded essentially the same results as did intact antibody (Table 1). However, recovery from Fab fragment-induced inhibition was slower than was recovery from intact antibody-induced inhibition. This was probably due to 1604 Fab fragments being more resistant to proteolytic activity than was intact 1604 antibody (data not shown). The requirement for higher concentrations of 1604 Fab fragments probably indicates that the affinity of mAb 1604 was low.

An alternative interpretation of the blocking effect of 1604 IgG or Fab fragments is that their interaction with an outer membrane protein either sterically hindered an interactive site or induced a secondary nonspecific alteration of a nearby site.

The cell surface antigen recognized by mAb 1604 was shown earlier to be present on vegetative and developing cells (20, 28). While the evidence suggests that the antigen has a developmental function, this antigen may also have vegetative functions. Whatever its specific role is, the action of 1604 antigen must be early in development—i.e., prior to aggregation (Fig. 3*A*). Because sporulation, as well as fruiting body formation, was inhibited (Table 1), the block would seem to occur before branching of the developmental pathway into aggregation and sporulation pathways (4).

These data are consistent with the hypothesis that 1604 antigen plays a role in contact-mediated cell interactions in *M. xanthus*.

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