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Role of *Myxococcus xanthus* Cell Surface Antigen 1604 in Development

BRUCE W. JARVIS† AND MARTIN DWORKIN*

Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455

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The inhibition of development of *Myxococcus xanthus* by monoclonal antibody (MAb) 1604 has been further investigated with two MAbs produced against the affinity-purified cell surface antigen (CSA) 1604. Both of these second-generation MAbs, 4070 and 4054, reacted with the same band at 150 kilodaltons (kDa) on Western immunoblots of lysed and reduced cells. This band was also identified by MAb 1604. However, the affinity-purified CSA was a complex of the two proteins (51 and 23 kDa) and lipopolysaccharide (LPS) that the 150-kDa material comprised. One of the three MAbs, 4070, reacted with LPS on Western immunoblots. Another MAb, 4054, reacted with the 23-kDa protein, and MAb 1604 reacted with the 51-kDa protein found in the CSA complex. Competitive binding studies verified that MAbs 4054 and 1604 identified different epitopes, and MAb 4070 probably reacted with a third epitope of the CSA 1604 complex. MAb 4054 blocked development, although not as thoroughly as MAb 1604 did, when added at 60 μg/ml to cells undergoing submerged development. In contrast, MAb 4070 prevented sporulation in submerged development and induced the cells to reaggregate in rings around the initial aggregation centers. A mutant strain of *M. xanthus* that is deficient in the epitope for MAb 1604 retained the epitope for MAb 4054. The affinity-purified antigen 1604, when added to cells at ≥550 ng/ml, altered the appearance of the fruiting bodies and at higher concentrations prevented fruiting body formation. The CSA 1604 moiety responsible for this inhibitory effect is apparently a peptide constituent and not the LPS.

One of the fundamental questions in cellular biology concerns mechanisms of cellular recognition. Little attention has been paid to those aspects of intercellular interactions that are mediated by cell-to-cell contact rather than by the exchange of diffusible, extracellular signals. This question has usually been addressed in eucaryotes, such as the cellular slime mold *Dictyostelium discoideum* (2) and in neuronal cells in culture (27). A considerable amount of recent work (4, 29) has demonstrated that these sorts of questions can appropriately be investigated in the context of the developmental cycle of the gram-negative soil bacterium *Myxococcus xanthus*. In response to nutrient loss, vegetative *Myxococcus* cells somehow identify one another during intermittent contact and swarm together into colonial aggregates that form fruiting bodies containing desiccation-resistant myxospores (20).

In our laboratory, Gill et al. (10, 13) have produced monoclonal antibodies (MAbs) against different antigens on the surface of whole cells of *M. xanthus* DK1622. These MAbs were produced against cells harvested at various times during development (10, 11). One of these, MAb 1604, when added as intact immunoglobulin or as Fab fragments, inhibited sporulation and fruiting body formation (12) but did not inhibit motility (unpublished observation). MAb 1604 was used to affinity-purify the cell surface antigen (CSA) 1604 complex, the concentration of which does not change substantially during vegetative growth and development (10). The CSA 1604 complex neutralized the inhibition caused by MAb 1604 (12). The immunoaffinity purification of and some of the properties of the CSA 1604 complex are described in the accompanying paper (16). Our intention in this paper is to examine in more detail the developmental effects of MAbs directed against the CSA 1604 complex and to examine the possible role of the antigen complex in mediating intercellular interactions in *M. xanthus*. We also suggest that the complex may play a role in cell adhesion.

* Corresponding author.

† Present address: Department of Biochemistry, Gortner Laboratory, University of Minnesota, St. Paul, MN 55108.

MATERIALS AND METHODS

Bacterial growth and enumeration. The procedures for growing, counting, and inducing the development of *M. xanthus* DK1622 (19) are described elsewhere (16).

Hybridoma growth and production and ELISA procedures. Hybridoma growth and enzyme-linked immunosorbent assay (ELISA) procedures were done by the methods of Gill et al. (13), Gill and Dworkin (10), Gill et al. (12), and Jarvis and Dworkin (16). MAb 1604 (IgG2a) was produced by immunizing mice with whole *M. xanthus* cells (10). MAbs produced against affinity-purified CSA 1604 were designated second-generation MAbs. Two of these second-generation MAbs, 4054 and 4070, are described in this paper. MAb 4054 is an IgG2a and 4070 is an IgG2b, as determined by ELISA reactivity with a MonoAb-ID EIA Kit (Zymed Laboratories, San Francisco, Calif.). The second-generation MAbs were produced by immunizing a BALB/c Wat (University of Minnesota colony) female mouse with 100 μg of CSA 1604 by subcutaneous injection. The CSA had been emulsified by sonication in an equivalent volume of Freund complete adjuvant (Sigma). One month later, 100 μg of CSA 1604 was similarly emulsified in Freund incomplete adjuvant (Sigma) and injected into the mouse. One month after the second immunization, the mouse serum was found to have a high concentration of antibody reactivity against whole *M. xanthus* cells (ELISA titer of 1:1,024). The mouse was then "boosted" with an intravenous injection of 50 μg of CSA 1604 into the tail vein. Three days later, the spleen was excised for the fusion procedure of Gill and Dworkin (10).

Western immunoblots of lysed cells probed with MAbs. The procedures for gel electrophoresis, electroblotting, and

probing Western immunoblots with MAbs were as described by Gill and Dworkin (10). Lysed cell suspensions were made from cells grown in tissue culture flasks as described by Jarvis and Dworkin (16).

Spot immunoblots of whole cells. For spot immunoblots, the methods of Fink et al. (7) were followed with DK1622 as a reference strain and JZ8900 as a mutant strain deficient in the epitope for MAb 1604. Two independent transductants, numbered 3 and 5, of JZ8900 were tested.

CSA purification. The methods for lysing cells by osmotic shock, extracting the CSA 1604 by an immunoaffinity column of MAb 1604, and purifying the high-molecular-mass fraction of the CSA by gel filtration are described by Gill et al. (12) and by Jarvis and Dworkin (16).

LPS purification and Western immunoblots. Lipopolysaccharide (LPS) from vegetative cells of *M. xanthus* was extracted and purified by the method of Panasenko (26). LPS (4 μg) was electrophoresed, transferred to nitrocellulose, and probed with MAbs by the method of Young and Davis (34).

Additions of MAb, CSA, and LPS to submerged-development cultures. MAbs were purified from ascites fluid (1604 and 4054) or from hybridoma culture (4070) on protein A-Sepharose CL 4B (Pharmacia, Uppsala, Sweden) as described by Ey et al. (6). The MAb solutions were then dialyzed against 10 mM MOPS [3-(*N*-morpholino)propanesulfonic acid, pH 7.20] with 50 mM NaCl and filter-sterilized, and the protein concentration was determined by the bicinchoninic acid method (31). A volume (<5 μl) of MAb solution containing 6 μg of protein was added at intervals to cells submerged under 100 μl of MOPS-salts (12) buffer in each well of a 96-well plate. Similar results have been obtained with 3 μg of MAb per addition.

Immunoaffinity- and gel filtration-purified CSA 1604 complex (16) was dialyzed against MOPS-salts buffer at 4°C, and the residual Zwittergent 3-14 was adsorbed onto Amberlite XAD beads (Sigma) for 15 min with shaking (23). After the highest concentration of CSA was so treated, the solution was aspirated from the beads, and serial twofold dilutions were made in volumes sufficient for duplicate determinations.

Proteolysis of the CSA 1604 complex was carried out in 20-μl volumes of CSA 1604 solution that had been dialyzed and adsorbed as above. A 1-μg amount each of trypsin, subtilisin, and proteinase K (all from Sigma) was added to the CSA in MOPS-salts buffer with 0.05% sodium dodecyl sulfate (SDS). The mixture was incubated at 32°C for 2 h, dialyzed at 23°C against MOPS-salts buffer to remove SDS, and diluted 22-fold before use.

LPS was quantitated by weighing the lyophilized powder obtained after purification. The LPS was then diluted in distilled water to the desired concentration and added to cells undergoing submerged development.

Covalent conjugation of enzyme to MAbs. The conjugation of the enzyme horseradish peroxidase (HRP) to MAbs involved four steps as described by Engvall (5): activation, dialysis, coupling, and blocking. The HRP (2.5 mg; Sigma) was activated in 50 μl of 100 mM sodium phosphate (pH 7.2)–100 mM NaCl–0.05% Tween 20–1% bovine serum albumin (BSA)–0.02% azide–1.25% glutaraldehyde (Sigma) at 23°C overnight. The next morning the mixture was diluted with 100 mM sodium carbonate (pH 9.2) and dialyzed against the carbonate buffer for 8 h at 23°C in Spectrapor 4 tubing (Fisher Scientific, Chicago, Ill.). Simultaneously, 80 μl (250 μg) of protein A-Sepharose-purified MAb was added to 120 μl of the carbonate buffer described above and also dialyzed against the carbonate buffer. After dialysis, 500 μg of the HRP-glutaraldehyde was coupled to 250 μg of MAb by mixing the two together and incubating them overnight at 23°C. The unreacted glutaraldehyde crosslinking groups were then blocked by adding 5 μl of 200 mM lysine (Sigma) in carbonate buffer. The MAb-HRP conjugates were stored in 50% glycerol at 4°C with 0.005% Thimerosal, a bacteriocide.

Competitive binding of MAbs to cells. Cells were grown in 96-well plates as described elsewhere (16). The plates were dried at 23°C and stored at 4°C. To test whether an MAb competed with another MAb for binding to cell surface epitopes, the MAb-HRP was first titrated in duplicate against the cells. An optimal dilution of MAb-HRP was determined which gave ELISA A_{490} readings of approximately 1.00. Except where noted otherwise, all buffers used herein were those for the ELISA procedure. In subsequent experiments, the potentially competing MAbs were diluted in duplicate wells, mixed with an equal volume (50 μl) of the MAb-HRP at its optimal dilution, and incubated for 1 h at 37°C. The wells were washed three times, and 100 μl of the substrate solution was added for a 15-min incubation in the dark. The substrate solution was 4 mg of *o*-phenylenediamine (Sigma) dissolved in 10 ml of 100 mM citrate-phosphate (pH 5.0) plus 1 μl of hydrogen peroxide (Sigma). Then, 50 μl of 4 N sulfuric acid was added per well after the incubation and before the A_{490} was read. All readings were corrected for background absorbance values with wells containing only cells and substrate solution.

RESULTS

Production of second-generation MAbs. MAb 1604, a first-generation antibody produced in mice immunized with whole *M. xanthus* cells, blocked aggregation and fruiting body formation (12). Second-generation MAbs were produced by immunizing mice with the affinity-purified CSA 1604 complex. Second-generation MAb clones were screened for recognition of *M. xanthus* cells. From a total of 300 wells containing limit-diluted hybridomas, 71 wells had reactivity against intact cells during ELISA screening. Out of these 71 wells, eight second-generation MAbs were obtained that reacted with intact cells but not with purified LPS. MAbs reacting with CSA 1604 epitopes on the outside of the cell were identified by reactivity with intact cells. Alternatively, MAbs reacting only with epitopes exposed to the periplasmic space would have been identified by differential screening with intact and lysed cells, but no MAb was found that reacted only with lysed cells. Of the eight MAbs, only five were usable. Two of these five MAbs, 4054 and 4070, have been studied in depth. The three unusable MAbs included one IgM and one IgG1, neither of which was purifiable on protein A-Sepharose. The hybridoma cells producing the third unusable MAb grew too slowly to work with efficiently.

Western immunoblots. The reactivities of MAb 4054 and 4070 on Western immunoblots of lysed cells were identical to that of MAb 1604 (Fig. 1A). Under reducing conditions and with boiling, a 150-kDa band was identified by each of the three MAbs. (The bands would have been at 200 kDa without boiling [16].) These results suggest that the first- and the two second-generation MAbs all identify the same antigen complex.

Figure 1B contains Western immunoblots showing that MAb 4070 but not 4054 reacted with 4 μg of LPS that was present in the affinity-purified CSA 1604 complex. The

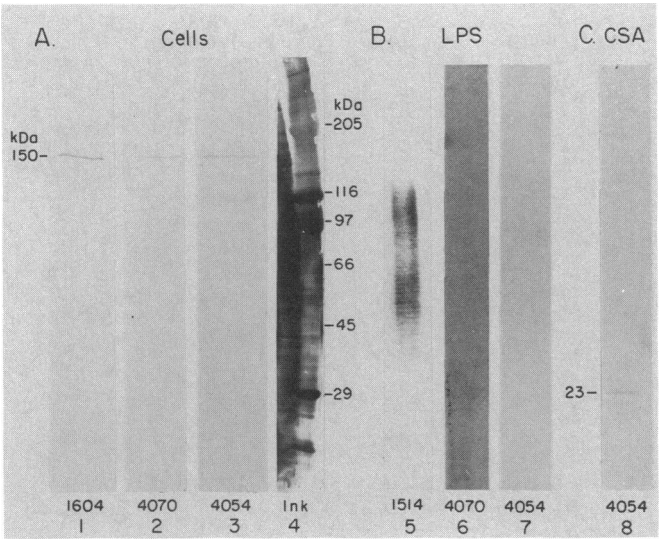

FIG. 1. (A) Western immunoblots (from reducing gels) of 2×10^8 *M. xanthus* cells per lane probed with MAbs 1604, 4070, and 4054. MAb 1604 is a first-generation MAb produced in mice immunized with whole cells. MAbs 4070 and 4054 were produced in mice immunized with the affinity-purified CSA 1604 complex. These two MAbs identified the same band at 150 kDa as that identified by MAb 1604. (B) Western immunoblots of 4 μg of *M. xanthus* LPS per blot were probed with MAbs 1514, 4070, and 4054. The blots show that the second-generation MAb 4070 but not 4054 reacted with LPS. MAb 1514 reacted with LPS, as expected. (C) Western immunoblot (from a nonreducing gel) of the affinity-purified CSA 1604 complex probed with MAb 4054. MAb 4054 reacted with the 23-kDa protein of the CSA complex.

reactivity of MAb 4070 with LPS (between 10 and 35 kDa) was weak, which may explain why the 4070 reactivity with LPS was not detected during three earlier ELISA screenings of the hybridoma clones. MAb 1514, which reacted with an O antigen epitope (8, 10), reacted with LPS, as expected. Neither MAb 4054 (Fig. 1B) nor MAb 1604 (16) reacted with LPS. Therefore, MAb 4054 probably also reacted with a CSA protein epitope, as did the original MAb 1604.

In Fig. 1C there is evidence from a Western immunoblot of a nonreducing gel that MAb 4054 reacted with an epitope on the 23-kDa protein of the CSA complex. Neither MAb 1604, which reacted with the 51-kDa protein (16), nor MAb 4070 (unpublished observation) reacted with the 23-kDa protein.

Competitive binding of MAbs to whole cells. MAbs 1604, 4054, and 4070 recognized the CSA 1604 complex (Fig. 1). To verify that these three MAbs identified different epitopes, competitive binding studies were done. In competitive binding studies in the presence of a competing antibody, the ELISA absorbance decreases. In the presence of a noncompeting antibody, the absorbance remains unchanged. Neither MAb 4054 nor 1604 competed with the binding of the other (Fig. 2A and B). The binding of each MAb-HRP was, however, competed by the homologous MAb. MAb 159-18 was a negative antibody control against a heterologous viral surface glycoprotein. MAb 159-18 determined the amount of absorbance expected from no competition.

From the data in Fig. 2, it can be concluded that MAbs 1604 and 4054 are definitely recognizing distinct epitopes. Based on the one-way competitive binding assay, MAb 4070 may bind to a third epitope on the CSA 1604 complex, but one cannot be certain from these data. It was not possible to test MAb 4070-HRP for competition with the other antibodies, because even the negative-control MAb 159-18 competed with it. We believe that this result means that MAb 4070-HRP had a very low binding affinity. Both MAb-HRP conjugates alone gave ELISA absorbance values similar to the highest absorbance values obtained during the competitive-binding assay, indicating that those high values represented no competition. It had been shown previously (unpublished observation) that the competing antibodies were present at saturating concentrations. Thus, lack of competition was not due to lack of complete occupation of the binding sites. During each ELISA, nonspecific binding of the antibodies was quantitated and subtracted from the other readings.

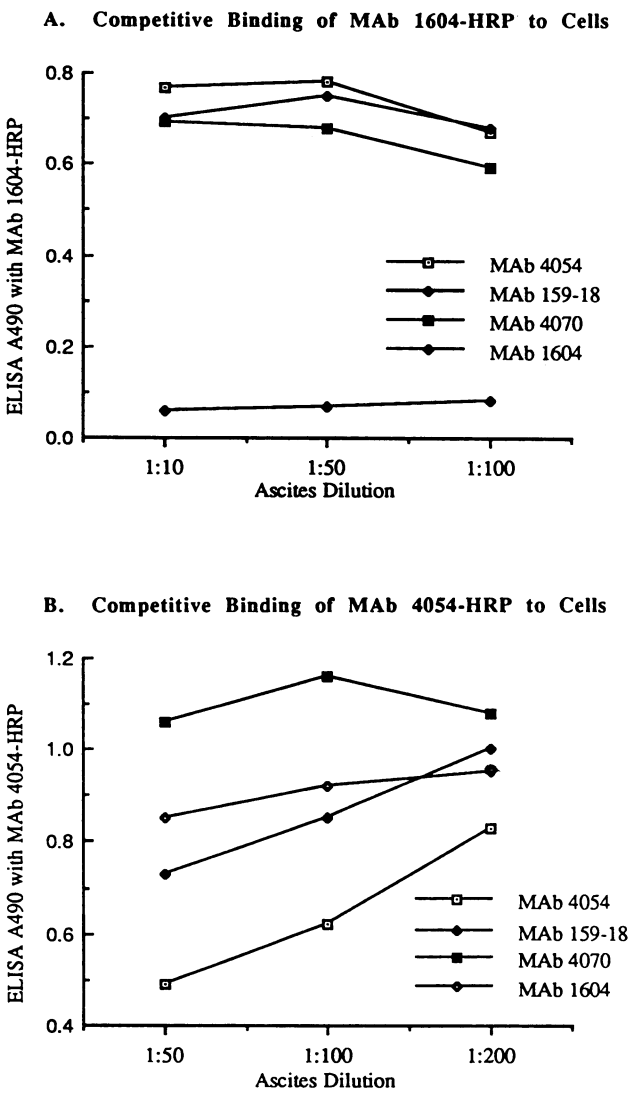

FIG. 2. Binding of MAb-HRP conjugates to 10^7 *M. xanthus* cells in the presence of competing and noncompeting MAbs. ELISA A_{490} values from binding of the MAb-HRP conjugate were plotted against dilutions of the potential competitor. Two conjugates were tested: (A) MAb 1604-HRP and (B) MAb 4054-HRP. Absence of competition produced high ELISA absorbance. Competition decreased the absorbance values.

Serial additions of MAbs to submerged-development cultures. Both the original MAb 1604 and the second-generation MAbs 4054 and 4070 perturbed submerged development, but

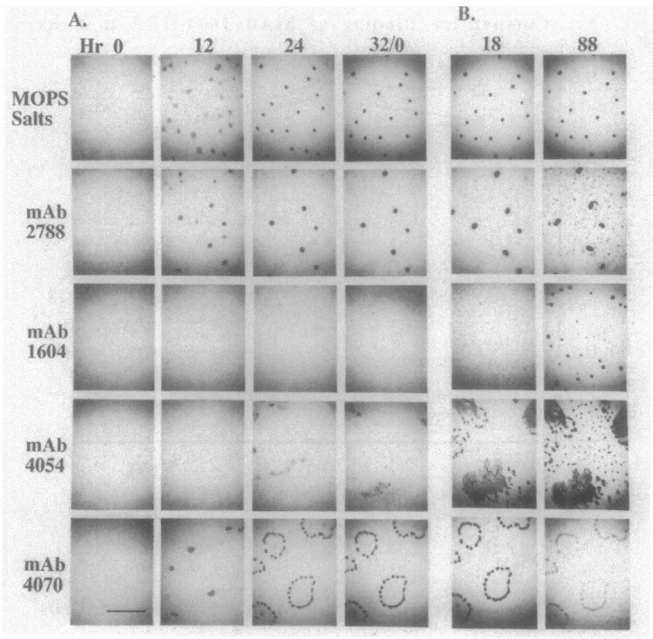

FIG. 3. Serial additions of four MAbs to developing *M. xanthus* cells. The horizontal axis is the time in hours (A) after onset of starvation and (B) after the last MAb addition. The vertical axis is a description of the substances added during starvation. MAb 1604 was the original antibody produced against whole cells. MAbs 4054 and 4070 were produced against the affinity-purified CSA 1604 complex. MAb 2788 was a negative control for the nonspecific effects of MAbs on development in submerged culture. (A) Antibodies (6 μg in a few microliters) were added into the 100 μl of MOPS-salts in each well at 4, 8, 12, 14, 24, 28, and 32 h after onset of starvation. The buffer control for normal development was the MOPS-salts buffer. (B) Recovery of *M. xanthus* after serial additions of the four MAbs. Hour 0 of recovery was timed from the last addition of antibody at 32 h after the onset of starvation. Bar, 1 mm. The numbers of viable spores per milliliter after 88 h of recovery were: for the MOPS-salts control, 1.2×10^7; for the MAb 2788 control, 1.4×10^8; for MAb 1604, 1.4×10^8; for MAb 4054, 1.5×10^8; and for MAb 4070, $<10^3$.

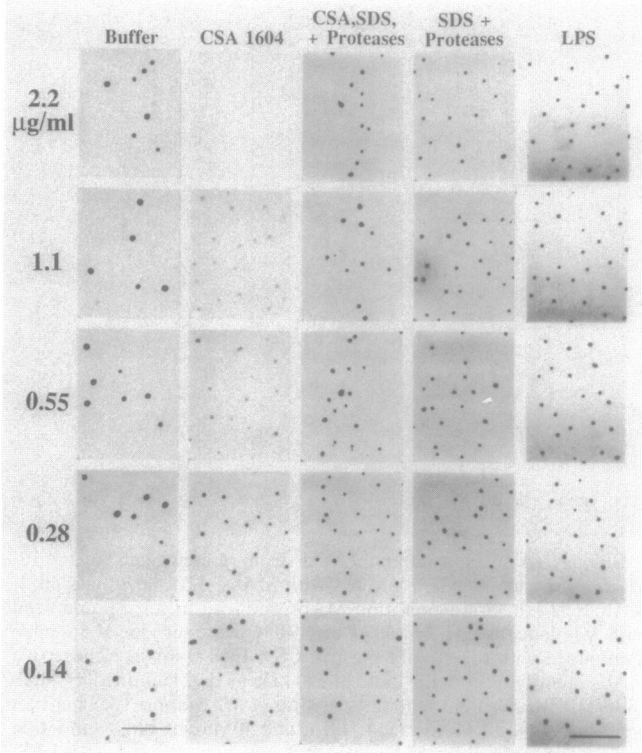

FIG. 4. Single addition to developing *M. xanthus* cells of twofold dilutions of buffer, the CSA 1604 complex, CSA 1604 complex proteolyzed in the presence of SDS, proteases plus SDS, or LPS. The horizontal axis is a description of the substances added to developing cells. The vertical axis is twofold serial dilutions of the substances added. The CSA buffer control contained 25 mM Tris (pH 7.5), 25 mM NaCl, and 0.01% Zwittergent 3-14 diluted into MOPS-salts in the same manner in which the CSA 1604 was prepared. The CSA was preincubated for 2 h at 32°C with or without the SDS and proteases. Then the SDS was removed from the mixtures by dialysis and adsorption with Amberlite XAD beads. The SDS and proteases mixture without the CSA was prepared similarly. Purified LPS was added exogenously. Bar, 1 mm.

in different ways. As depicted in Fig. 3, MAb 1604 at 60 μg/ml reversibly inhibited aggregation when added at 4, 8, 12, 14, 24, 28, and 32 h to developing cells in submerged culture. During recovery, the fruiting bodies were fully formed by 88 h after the last MAb 1604 addition. These results are in agreement with those of Gill et al. (12). The second-generation MAb 4054 also inhibited aggregation, although not as completely.

In contrast, MAb 4070, another second-generation MAb, did not prevent fruiting body formation but did disrupt fruiting body sporulation and pattern formation. By 12 h of development, larger than normal aggregates formed. They dispersed subsequently, and by 24 h smaller secondary aggregates reformed in a ring around the original aggregation centers (Fig. 3). At 18 to 28 h after the last MAb addition, tertiary aggregates began to form away from the rings. The rings of aggregates were seen to darken and then to become lighter again by 88 h of recovery from the last MAb addition. This translucence suggested that sporulation was incomplete. The viable spore counts verified that sporulation was reduced by at least four orders of magnitude (Fig. 3 legend). The negative controls in Fig. 3 were the additions of MOPS-salts buffer and of MAb 2788 (IgG2b) in MOPS-salts buffer. Both additions produced normal fruiting body development.

Effect of a single addition of the CSA 1604 complex on submerged development. It has been shown that two antibodies, 1604 and 4054 (both directed against protein epitopes of the CSA 1604 complex), inhibited developmental aggregation (Fig. 3). The antigen complex also altered development but during aggregation. The CSA 1604 complex at a concentration of ≥550 ng/ml, when added at 0 h to submerged-development cultures, altered the appearance of the fruiting bodies (Fig. 4). The fruiting bodies that formed did contain viable spores. At higher concentrations, up to 2.2 μg/ml, the CSA prevented fruiting body formation. The addition of as little as 140 ng of CSA per ml did not affect development.

Proteolysis of the CSA 1604 complex by a mixture of trypsin, subtilisin, and proteinase K in 0.05% SDS before addition of CSA to the cells reversed the alteration of development caused by the CSA 1604 complex (Fig. 4). The protease-SDS mixture without the CSA had no effect on development. An addition of 2.2 μg or less of BSA per ml did not prevent submerged development; 300-fold more BSA was needed to alter development (unpublished observation). These results suggested that the protein moiety of the protein-LPS complex inhibited development. To eliminate the possibility of LPS involvement, the following experiments were done.

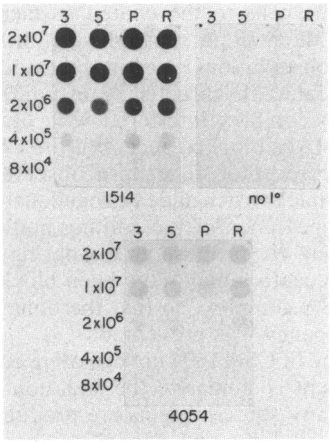

FIG. 5. Spot immunoblots of dilutions of *M. xanthus* cells from reference and mutant strains. Equivalent numbers of cells per spot were placed in horizontal rows. Vertical columns of spots contained, left to right: independent transductants 3 and 5 of JZ8900, the strain deficient in epitope 1604; cells (P) not pertinent to this work; and the nonmutant reference (R) strain DK1622. Immunoblots were probed sequentially with MAb 1514 or 4054, antibody conjugate, and then substrate solution or with only antibody conjugate (panel no 1^0) and substrate solution. The spots on the no 1^0 immunoblot in the rows with $\geq 10^7$ cells were due to the yellow pigment from the cells. In contrast, the spots on the other two immunoblots were due to the pink to blue color reaction of the substrate solution.

Effect of a single addition of LPS on submerged development. LPS purified from *M. xanthus* was also added to submerged-development cultures. LPS at ≤ 2.2 μg/ml, when added exogenously, did not alter submerged development (Fig. 4). However, CSA 1604 at 2.2 μg/ml altered fruiting body formation. Even at approximately eight times that amount of LPS (17.6 μg of LPS per ml), exogenously added LPS did not affect development (unpublished observation). Thus, the purified LPS per se did not account for the observed inhibition of submerged development. These data strengthen the argument that it is a protein moiety of the CSA complex that disrupted development.

Reactivity of MAb 4054 with mutant cells deficient in epitope 1604. Figure 5 shows spot immunoblots of nonmutant reference cells (DK1622) and cells deficient in the epitope for MAb 1604 (transductants 3 and 5 of JZ8900). MAb 1514 reacted as expected with all of the mutant and reference strains on spots of as few as 4×10^5 cells. Immunoblots (labeled no 1^0) exposed only to the antibody conjugate and to substrate solution showed no reactivity. MAb 4054 reacted both with reference cells and with as few as 2×10^6 cells of the mutant deficient in epitope 1604. Cells in the column marked P are not pertinent to this work.

DISCUSSION

Production and screening of second-generation MAbs. The second-generation MAbs were produced for three reasons. First, we hoped to find a second-generation MAb that blocked development as the original MAb 1604 did, thereby confirming the specificity of the effect of MAb 1604 on the cells. If all of the second-generation MAbs had the same effect as the original 1604, this would suggest that the effect is nonspecific, that is, not due to the unique ability of each MAb to bind to a different epitope. Second, if MAbs recognizing CSA epitopes exposed to the periplasmic space could be found, then some domain of the CSA 1604 complex would be transmembrane. Third, with a collection of MAbs recognizing various epitopes on the CSA 1604, it would eventually be possible to map the CSA (1, 9, 28) to distinguish which epitopes are involved in contact-mediated cellular interactions.

There are two possible explanations why all eight of the second-generation MAbs reacted with both intact cells and lysed cells during the ELISA screening. That is, none of the MAbs reacted only with lysed cells. The first possibility is that part of the CSA is exposed inside the periplasmic space or in the transmembrane region, but the antibodies failed to recognize this domain of the antigen. There are several reasons why antibodies may not have been produced against a putative antigen domain in the periplasm or transmembrane region. These epitopes may have been denatured by the combination of cell lysis and drying on the plastic surface (17, 30), or the putative transmembrane region may consist of highly conserved domains which are not immunogenic in mice (18). The second possibility is that the CSA is a peripheral membrane protein complex and has no transmembrane region. In this case, the CSA may be attached to the outer membrane by an interaction with LPS. The copurification of LPS with the CSA proteins and the location of the immunogold particles on the cell (16) both suggest that the CSA is a peripheral membrane protein complex.

Serial additions of MAbs to cells undergoing submerged development. MAb 1604 has previously been shown to halt development before aggregation (12). In addition, it has been shown that preincubation of the CSA 1604 with MAb 1604 neutralized the inhibition caused by the MAb (12). The inhibition of development by MAb 4054 suggested that, as is believed to be the case with MAb 1604, MAb 4054 bound to and covered another epitope (Fig. 1 and 2) of the CSA 1604 complex that may be involved in intercellular contact during development. In the presence of either of these two MAbs, crucial contact with the putative antigen receptor may be blocked and development may be halted before aggregation can occur. The finding that MAbs 1604 and 4054 but not 4070 gave similar results when added to submerged-development cultures provides further evidence that the CSA 1604 complex is actually involved in some developmental interaction among the cells. If the effect of addition of MAb 1604 were duplicated by all of the second-generation MAbs, one would suspect nonspecific effects from antibody addition. This seems unlikely, because MAb 4054 affected development differently than 4070 did.

The addition to 2×10^7 cells of 3 μg of MAb 1604 every 4 h has been found to block development effectively (D. Clemans, personal communication). The amount of MAb 1604 needed to saturate 99% of the CSA 1604 sites can be found by multiplying the K_d value of 9.1 nM (16) by 99 (3). For 2×10^7 cells, this value is 1.5 μg. Gill and Dworkin have shown that during development, *M. xanthus* is continually producing proteases that degrade added MAb (J. S. Gill and M. Dworkin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, I-130, p. 202). In addition, a 4-h exposure of MAb 1604 (60 μg/ml) to cells attached to a 96-well plate decreased the subsequent ELISA binding intensity of that MAb by 25% in comparison with ELISA intensities after control incubations without cells (unpublished observation). The amount of MAb 1604 bound by the cells was negligible (0.01%). This 25% decrease as a result of proteolysis would account for the necessity of adding twice the amount of antibody needed to saturate the antigen sites.

In contrast to MAb 1604, MAbs 4054 and 4070 bind to different domains of the CSA 1604 complex (Fig. 1 and 2)

(16) and thereby alter sporulation and fruiting body pattern formation in a different fashion (Fig. 3). Similarly, Gabay and Schwartz (9) found that four MAbs produced against the LamB maltose transport protein of the *Escherichia coli* outer membrane did not behave identically. Two of these four MAbs inhibited maltose transport; two did not.

The most notable aspect of the effect of adding MAb 4070 is that the cells seem to be prevented from terminating their development by sporulating and forming fruiting bodies. Instead, the cells continue to form new centers of aggregation. During normal development in submerged culture as carried out in our laboratory, some cells lyse (32, 33), and the survivors form about 10^7 viable myxospores per ml (12). (We are aware of the alternative interpretation for these events observed during development [25].) In contrast, the additions of MAb 4070 seem to prevent lysis and sporulation and instead reset the developmental program so that swarming and aggregation begin anew. For example, at 12 h of development (Fig. 3), the cells formed large aggregation centers, which then broke apart. The cells evidently swarmed away, and by 24 h reformed new centers in "Hexenringe" around the 12-h centers. The number of viable spores per ml after 88 h of recovery from the last MAb 4070 addition was less than 10^3 spores per ml (Fig. 3 legend), in contrast to $>10^7$ viable spores per ml formed under conditions of normal development (12).

In the presence of MAbs 1604, 4054, and 2788, more than 10^8 viable spores per ml were produced (Fig. 3 legend). The antibody additions apparently fed the vegetative cells, generated further growth and cell division, and produced more spores. The total amounts of MAb protein (180 μg/ml) added before aggregation (ca. 12 h of submerged development) were about the same as the amount of proteolyzed protein (casitone) in CF agar (150 μg/ml). One milliliter of CF agar (14) has enough proteolyzed protein and pyruvate for *M. xanthus* cells to grow into more than 100 colonies of 10^6 cells each and then to develop into fruiting bodies. Thus, the 10-fold-greater number of spores after MAb addition was probably due to the added protein.

Active moiety of CSA 1604. The addition of purified CSA 1604 complex to developing cells inhibited fruiting body formation (Fig. 4). The CSA contained both protein and carbohydrate, most likely in the form of LPS (16). Nevertheless, LPS, when purified and added exogenously, did not alter development (Fig. 4). However, proteolysis of the affinity-purified CSA did abolish the ability of exogenously added CSA to inhibit development. Thus, a protein moiety and not the LPS moiety of the CSA was probably interacting with the cells.

Mutant deficient in epitope 1604. A report has recently described a mutant of *M. xanthus* that has 4% or less reactivity with MAb 1604 and yet forms normal fruiting bodies under conditions of submerged development (7). We have shown that two independent transductants of this mutant strain JZ8900 reacted strongly with MAb 4054 (Fig. 5). Therefore, the mutant strain contains the domain of the CSA 1604 complex on which the 4054 epitope is located. Thus, there is as yet no evidence to contradict the findings which suggest that the CSA 1604 complex plays a role in development.

Cell adhesion. Our present working hypothesis is that one or more domains of the CSA 1604 complex bind to a putative receptor and thus mediate cell adhesion during early development. This is based on three observations. First, the CSA complex, when immobilized by the method of Lagenauer and Lemmon (21) on nitrocellulose-coated wells, mediated adhesion of the cells to the coated surface. Control wells coated with buffer alone showed very little cell adhesion (unpublished observations). Second, the addition of any one of three different MAbs directed against different epitopes of the CSA 1604 complex interfered with development. Presumably the MAbs blocked access of the CSA 1604 domain to its putative receptor. The third observation is that the CSA 1604 complex, when added exogenously at ≥550 ng/ml, altered the appearance of the fruiting bodies or prevented their formation. We propose as a working hypothesis that the putative receptors may have been blocked by the addition of the CSA complex, so that the bona fide function of receptor occupancy was prevented.

The presence of CSA 1604 in vegetative cells and throughout development is similar to the situation in *Bacillus subtilis*, where many spore proteins are produced hours before they are assembled into the spore (22). Also, protein S of *M. xanthus* is produced and retained in the cytosol of vegetative cells and is translocated to the surface only late in development (15, 24). While it is not yet possible to determine whether the CSA 1604 complex is essential for development, the data clearly point to its playing an important role in the process.

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