rent isolates from swine. This raises the question as to how these viruses were introduced into the birds. In 1975, when serologic evidence of swine viruses in turkeys was first obtained (*10*), sick pigs on the same farm were suspected as the source. Since swine viruses can infect turkeys, it is feasible that such transmission occurred. More recently, an outbreak of influenza in turkeys in Colorado involved confined birds with no known contact with pigs. It was suggested that humans were the source, having acquired the viruses while slaughtering pigs (*11*). Since swine viruses infect humans (*1*), it is possible that infected farm personnel did transmit the viruses to the turkeys.

The reverse is also possible—that is, that the turkeys transmit these viruses to other hosts, particularly humans in close contact with them. The infection of the laboratory technician by one of the turkey isolates establishes the potential for such an event. Whether such transmission occurs in nature is not known; however, in cases of human infection with swine viruses, epidemiologists should consider turkeys as well as pigs as a potential source.

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Mycoplasma-Like Organisms: Occurrence with the Larvae and Adults of a Marine Bryozoan

Abstract. *Larvae and adults of the marine bryozoan* Watersipora cucullata *invariably possess numerous extracellular mycoplasma-like organisms. Mesodermally encapsulated groups of these atypical bacteria occur in the visceral coeloms of all colony members. In contrast, thousands of the symbionts are externally attached to each larva along a unique superficial groove; the microorganisms are internalized during the complex metamorphosis, thus inoculating the incipient colony. The consequences to the bryozoan of this association are not known.*

Symbiotic associations of prokaryotes with the digestive tracts of various marine invertebrates are widespread in occurrence. Most of these associations involve Gram-positive or Gram-negative eubacteria. Comparatively little is known, however, of the diversity and significance of nonpathogenic associations of prokaryotes with other organs of marine invertebrates (*1*). We report here the occurrence of mycoplasma-like organisms with the larvae and adults of a marine bryozoan *Watersipora cucullata* (*2*) and describe the transfer of these symbionts from the larva to the progenitor and successive, asexually produced individuals of the adult colony. Mycoplasma-like organisms are well known from vertebrates, insects, and plants (*3*) but have seldom been observed in marine invertebrates (*4*).

Colonies of *W. cucullata* were collected in April 1969 and from March through July 1982 from four localities along the southern California coast (*5*). Adults and larvae have many pleomorphic prokaryotic cells that lack discernible cell walls (*6*). These cells vary in size, usually from 3 to 6 μm in diameter, but some are as large as 14 μm. Their shape, as determined from reconstruction of 1-μm sections, examination of living cells, and by scanning electron microscopy, is globular with one or several large lobes (Fig. 1, A and C). The finely granular cytoplasm is virtually free of organelles, although the cells frequently contain a central, spherical, electron-opaque body that may be ribosomal. The cells lack a discrete nucleoid region, but wisps of DNA are seen in some sections. The entire cells fluoresce intensely in living and Formalin-fixed preparations after staining with DAPI (Fig. 1B), indicating the presence of DNA (*7*). The plasma membranes lack a discernible extracellular coat and, frequently, occur in apposition to the membranes of adjacent cells. Identification of the symbiont can only be tentative at this time because initial attempts to isolate and culture the cells have not been completed. Morphological evidence suggests that the cells are mycoplasms or L-form bacteria. We refer to them here as mycoplasma-like organisms (MLO's).

In *W. cucullata* larvae, MLO's are found in a dense stratum lining the floor of a specialized latitudinal groove on the surface of the larva (Fig. 1C). The transformation from larva to adult results from a complicated sequence of morphogenetic movements that occurs in all known bryozoans (*8*, p. 91). The groove is situated between the corona (the ciliated larval locomotory organ) and the pallial sinus. The groove is approximately 22 μm deep and 17 μm high and encircles the larva except in the anterior midline where it is interrupted for about 20 degrees. The floor of the groove is formed by the most aboral portions of 28 to 30 of the 32 coronal cells. The MLO's are attached to elongate microvilli that project into the groove. Each larva may have hundreds to several thousands of the prokaryotes. The remaining portions of the groove are formed by a ring of approximately 200 biciliated, supracoronal cells which collectively form a flange that overhangs the groove as a roof. Although most components of the larval anatomy of this bryozoan can be considered homologous with structures in other bryozoan larvae (*8*, p. 97), the groove and flange have no known counterparts. They appear to be specializations of this larva and thus facilitate the transport of the symbiotic microorganisms from one adult generation to the next.

The larvae of a closely related species of bryozoan, *Cryptosula pallasiana*, which occurs sympatrically with *W. cucullata* and has a similar growth form and reproductive pattern, were also examined. The larvae of *C. pallasiana*, although comparable in morphology and provided with similar rings of coronal and biciliated supracoronal cells, lack a groove and flange and do not possess MLO's anywhere either on the surface or internally.

In *W. cucullata*, MLO's are transmitted from the surface of the larva to the interior of the first adult individual (the ancestrula) at metamorphosis. Early in metamorphosis the ciliated locomotory cells and other transitory larval tissues are directed to the interior of the metamorphosing individual. Once internalized, these tissues form a nutrient mass that supports the differentiation of the ancestrula from preformed anlagen (and sustains the early growth of the colony). The MLO's, which are still attached to the coronal microvilli, are internalized. Instead of being histolyzed with the transitory larval tissues, however, they are segregated into a number of small packets, each surrounded by one to several mesodermal cells (Fig. 1D). The distribution of the MLO's to the ancestrula and the first three asexually formed individuals of the young colony occurs when the nutrient mass (where the packets of MLO's are distributed) is partitioned between the four developing zooids. In later stages of colony growth, after the nutrient mass has been fully absorbed, packets of MLO's encapsulated by mesodermal cells are found associated with the funicular system and the coelomic lining of the visceral coelom of each individual in the colony. The packets are most often associated with the basal wall of an individual and with regions proximal to the pore plates between adjacent individuals. Packets of MLO's and strands of funicular tissue at the growing edge of the colony become incorporated into the marginal buds during asexual reproduction as transverse walls partition the budding zone. There is no evidence that MLO's occur except in packets within the adult or that they are transmitted through communication pores which connect adjacent individuals.

The MLO's within the adult are indistinguishable from those found on the surface of the larva. Evidence of reproduction of individual MLO's or of the packets has not been observed, but repeated multiplication of both is required to account for the large numbers of MLO packets within a colony. Other extra- or intracellular symbionts have not been found in either larval or adult tissues.

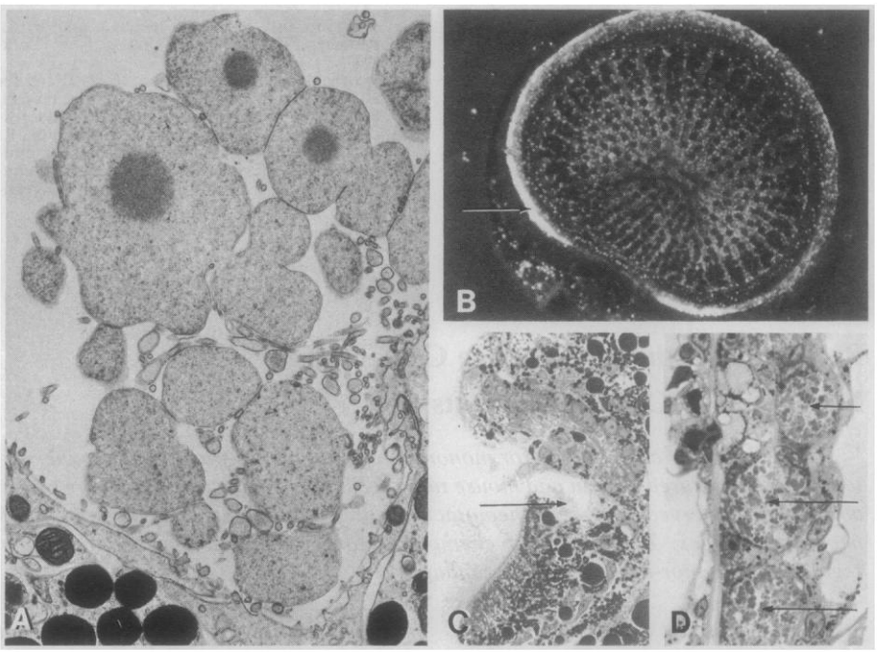

Fig. 1. (A) Transmission electron micrograph of several pleomorphic MLO's attached to microvilli of a larval coronal cell (×8510). (B) Fluorescence micrograph of a DAPI-stained larva in apical view. The bright band (arrow) is constituted of thousands of MLO's; the band is interrupted at the anterior midline of the larva (6 to 7 o'clock) and partially obscured by larval tissues from about 1 to 5 o'clock. The light dots represent nuclei of larval epidermal tissues; those of the large apical disk are in a complex radial pattern (×135). (C) Light micrograph of radial section of a larva with MLO's (arrow) in surface furrow; the floor of the groove is formed by part of the heavily ciliated coronal cell (×685). (D) Light micrograph with three capsules of MLO's (arrows) associated with a transverse wall in adult bryozoan; a pore plate interconnecting the two zooids is seen in the upper left quadrant (×907).

How MLO's are transmitted between generations is not fully understood. Each brooded embryo is encased within an acellular envelope throughout development. MLO's are not present within the envelope even in late embryos, which possess a fully formed, but empty, groove. MLO's are concentrated, however, in large packets adjacent to the brood space. Hatching from the envelope occurs at the time of larval release. For several minutes after its escape from the envelope, the larva remains tethered to the parent by a strand of mucus that extends from the orifice of the parent to the oral pole of the larva, originating from within the ciliated groove of the pyriform organ. Although the "loading" of the MLO's onto the larva has not been directly observed, it seems apparent that this must take place after rupture of the embryonic membrane during the few minutes while the larva is still tethered to the adult. Although this period is brief, each of the numerous free-swimming larvae had a rich complement of MLO's within the groove and, in all but a few cases, the microorganisms were distributed along the entire groove.

The nature of the interaction between the prokaryote and bryozoan is not known, but the MLO's are probably not pathogenic in that *W. cucullata* is a widely distributed species that is often dominant in fouling communities and we have found heavy infection in 100 percent of the samples taken.

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7. Living larvae of *Watersipora cucullata* were transferred to seawater containing DAPI (4′,6-diamidino-2-phenylindole) (2 to 5 μg/ml; Sigma) for periods of 30 seconds to 5 minutes. After being rinsed in a large volume of filtered seawater, larvae were prepared as wet mounts and examined by epifluorescence (Olympus Vanolux research microscope with filter) by the method of A. W. Coleman [*Limnol. Oceanogr.* **25**, 948 (1980)]. Photomicrographs (Ektachrome 400) were made of larvae killed with dilute Formalin.
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Regulation of the Macrophage Content of Neoplasms by Chemoattractants

Abstract. *Factor chemotactic for mononuclear phagocytes was found in supernatant fluids of cultured human and mouse tumor cells. In 11 mouse tumors there was a correlation observed between chemotactic activity and macrophage content of neoplastic tissues. Tumor-derived chemoattractants appear to participate in the regulation of tumor-associated macrophages.*

Macrophages are a major component of the lymphoreticular infiltrate of tumors in humans and mice, but their significance in vivo is largely a matter of speculation (*1*). How the macrophage content of neoplasms is regulated is not known. A relation between macrophage content of neoplastic tissues and tumor immunogenicity has been reported (*2, 3*), indicating that tumor-directed immune responses would be the major determinant of entry of blood monocytes into neoplastic tissue. However, some observations are not consistent with this view (*4*). Evans (*4*) chemically induced sarcomas in mice and found no relation between immunogenicity and macrophage concentration at the tumor site. Moreover, transplantation into nude or thymus-deprived mice (*4*) did not decrease tumor-associated macrophages (TAM's). Although in some systems specific immunity appears to play a role (*2, 3*), it is not the sole or most important determinant controlling TAM's. The possibility that macrophages accumulate in tumors attracted by neoplastic cells has been the subject of conflicting reports (*5, 6*). We found that neoplastic cells of mouse or human origin produced chemoattractants for macrophages and that there is a relation between production of chemotactic factors by tumor cells and the amount of macrophage infiltration in vivo.

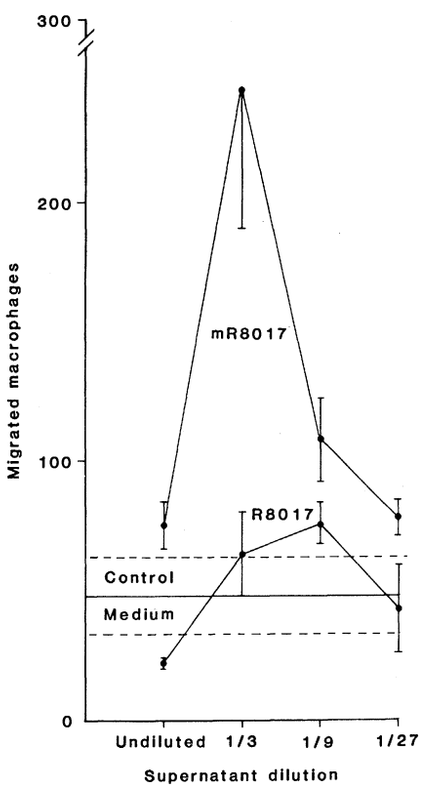

Fig. 1. Correlation between chemotactic activity and tumor-associated macrophages (TAM's) in mice. This is a typical dose-response experiment with supernatants from the low macrophage content (7 percent TAM's) sarcoma R8017 and its subline with a high macrophage content (20 percent TAM's) mR8017. Supernatants were harvested from subconfluent cultures (4.3×10^6 cells per 25-cm^2 flask for R8017 and 4.8×10^6 cells for mR8017).

Chemotaxis of human monocytes or mouse (male C57B1/6) macrophages was measured (*7*) in chemotactic chambers with 5-μm polycarbonate filters (3400253, Neuroprobe). Blood samples taken were from healthy human volunteers and mononuclear cells were separated by sedimentation at 400*g* for 20 minutes on Ficoll-Hypaque. Counts were made on cytocentrifuge smears, and cells were resuspended (1.5×10^6 monocytes per milliliter) in RPMI-1640 or minimum essential medium (MEM) with 10 percent fetal bovine serum (FBS). A mild inflammation was induced in mice by interperitoneal injection of 1 ml of phosphate-buffered saline, and after 24 hours peritoneal exudates were collected. Mouse peritoneal macrophages were resuspended (2×10^6 macrophages per milliliter). Chemotaxis was assessed after 1.5 and 4 hours of incubation at 37°C for human monocytes and mouse macrophages, respectively (Table 1). Cells were washed and seeded in RPM I-1640 or MEM with 10 percent FBS (1×10^5 cells per milliliter) in 25 cm^2 tissue culture flasks (5 ml). Supernatants, collected when cultures were subconfluents, were centrifuged at 600*g* for 15 minutes and tested immediately or stored at −20°C.

A summary of the chemotactic activity of supernatant fluids from various mouse and human tumor cells is presented in Table 1 and the results of typical individual experiments in Fig. 1. Supernatants of mouse carcinomas and sarcomas had appreciable chemotactic activity for mononuclear phagocytes. Production of chemoattractants was observed with long transplanted tumors (Lewis lung carcinoma; 1023, 3T3-B77, mFS6, M4, and M9 sarcomas), early passage chemically induced sarcomas (MN/MCA1, second and fourth transplant generation; mR8017, second and fourth transplant generation), and tumors obtained from autochthonous hosts (two spontaneous mammary carcinomas, R8018, R8016, and R8039). Chemotactic activity was observed with both in vitro established cell lines and with primary cultures (data not shown). The R8001 and R8017 sarcomas and the TLX9 and YAC-1 lymphomas showed no appreciable chemotactic

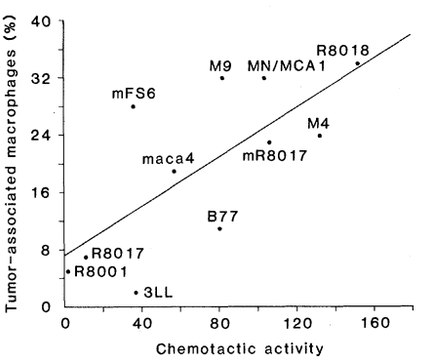

Fig. 2. Correlation between chemotactic activity in supernatants in vitro and tumor-associated macrophages in vivo. Chemotactic activity was calculated as the area under the dose-response curve after subtraction of migration with control medium (see Fig. 1) ($r = 0.71$, $t = 3.083$ (*10*), $P = .013$). We excluded the R8039 and R8016 sarcomas from the analysis because the percentage of TAM's in these tumors varied between experiments (Table 1); mammary carcinoma 1 was excluded as well because viability did not exceed 60 percent, making the TAM evaluation questionable. However, when these three tumors were included (taking the mean percentage of TAM's for the two sarcomas), a statistically significant correlation was still found ($r = 0.58$, $t = 2.49$ (*13*), $P = .028$).