



**Immunocompetence in the Lowest Metazoan Phylum: Transplantation  
Immunity in Sponges**

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brane is maintained for the entire period in which mean spike rate and burst rate are decreased, and these effects on bursting pacemaker activity can be duplicated by direct hyperpolarization of the cell soma. (ii) The peak increase in conductance associated with the SIP is large, amounting to  $61 \pm 15$  percent ( $N = 8$ ) of the normal membrane conductance measured at the cell soma. Correspondingly, the somatic action potentials are partially shunted during the SIP (Fig. 1B). (iii) The reversal potential of the early phase of the SIP is near the equilibrium potential for  $K^+$ , as would be expected for a  $K^+$  conductance increase located on or near the soma (15). These features of the SIP, and its smooth onset and prolonged duration, are consistent with the hypothesis that it is induced hormonally, rather than transynaptically, by a neurosecretory product released from bag cell axons in the sheath overlying the target cell somata.

The bag cells have two effects on bursting pacemaker neurons: inhibition, as described here, and augmentation, as described before (7). The question of how these multiple actions are mediated arises. Does one peptide, the egg-laying hormone, produce both of them or, among several alternatives, do the bag cells release more than one chemical messenger, each with its own effect on individual target neurons? Further studies addressing this question may provide new insights into the roles of peptides in naturally occurring interactions between neurons.

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11. A bipolar stimulating electrode was placed (on the right or left bag cell cluster) such that the cathode was as close as possible to the bag cell being recorded. The bag cells were activated

- with rectangular, constant-current pulses (0.2 msec, 2 to 28 mA, delivered at a rate of five per second). The stimulus intensity was gradually increased until it produced subthreshold depolarizations in the recorded bag cell without evoking synaptic inputs to other ganglion neurons. The bag cell burst was then initiated by a 1- to 2-second train of these pulses.
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  13. It also shows that the delay to onset of the response is about 1 second. The estimated latencies in unpolarized cells are much longer, 4 to 8 seconds. This difference may be more apparent than real, however, since the bursting pacemaker potential in unpolarized cells obscures the precise onset of the SIP and produces larger estimates of onset delay.
  14. In eight experiments, the mean time to peak was  $0.92 \pm 0.13$  minute ( $\pm$  S.E.M.).
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  16. Input conductance was still  $38 \pm 7$  percent

( $N = 4$ ) above baseline 30 minutes after the onset of the response.

17. In four experiments, the amplitude of hyperpolarization attributable to the second process reached a maximum of  $7.5 \pm 1.4$  mV at  $18 \pm 5.5$  minutes after the beginning of the bag cell burst. In some examples, in which the second process was sufficiently dissociated from changes in input conductance, the downward shift of the I-V line was associated with a secondary hyperpolarization of the cell. In Fig. 1B, a secondary hyperpolarization started about 2 minutes after the onset of the bag cell discharge. It is possible that this is due to the onset of the second process.
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## Immunocompetence in the Lowest Metazoan Phylum: Transplantation Immunity in Sponges

**Abstract.** *Isografts of Callyspongia diffusa fuse compatibly, but allografts are invariably incompatible. Extensive polymorphism of cell-surface histocompatibility markers is evident. The histocompatibility barriers range from strong to weak depending on the interclonal combination, but early rejection with conspicuous cytotoxic sequelae is typical. Reaction times of first-set, second-set, and third-party grafts indicate highly discriminating transplantation immunity with a specific memory component.*

Until recently, specific immune systems with selectively inducible responsiveness and a memory component were considered to be restricted to vertebrates. However, among higher invertebrates with diverse leukocyte-type cells, unequivocal immunorecognition at the allogeneic level has been reported in diverse worms (1), echinoderms (2), and tunicates (3). At the lower invertebrate level of coelenterates, allogeneic incompatibilities under controlled laboratory conditions are now documented for hydrozoans and gorgonians (4), as well as sea anemones (5) and corals (6). A specific alloimmune memory component of transplantation immunity has been demonstrated in representatives of three phyla: echinoderms, annelids, and coelenterates. To find among corals and now sponges the essential attributes of adaptive immunity (7) in a sharply discriminating form is surprising. Occurrence of specific allograft rejection at this lower level of phylogeny could represent the origins of both cell-mediated immunity and of the major histocompatibility complex of higher vertebrates.

Interspecific tissue incompatibility has long been known in sponges. When cell suspensions from two different species are mixed, each resulting aggregate appears to be composed of cells of one of the species alone (8). However, neither cell adhesion nor cell aggregation is strictly intraspecific among many

sponges (9). Incompatibilities in mixed aggregation experiments do not usually lead to cell death, but this could reflect tests of insufficient duration (1 to 2 days) or loss of immunocytes during tissue disruption and cell processing. Several distinctive types of potential immunocytes known as amoebocytes, including some capable of efficiently phagocytosing foreign particles and cells, are found in intact sponges (10). Early interspecific rejection reactions have been observed in contact zones between intact branches of *Microciona* and *Haliclona*, but no incompatibility was discerned between allogeneic *Microciona* grafts in experiments of 3 weeks duration (11). The latter result in light of our findings is probably attributable to immunosuppression at low temperatures. Intra-specific incompatibility was first recorded among separate strains or individual sponges of the freshwater species, *Ephydatia fluviatilis*. Incompatibility in contact zones was manifest by a discrete border separating allogeneic sponges in contrast to control intrastrain grafts which fused or merged compatibly (12). No cytotoxic reactions or other sequelae of rejection were noted in conjunction with allogeneic nonfusion.

Colonies of the large, ramose species of the tropical Indo-Pacific sponge *Callyspongia diffusa* exist in several different locations in Kaneohe Bay, Hawaii. Fully viable *Callyspongia* exhibit a uniform

Table 1. Evidence of specificity and memory in alloparabiont reaction times of *Callyspongia diffusa*.

Series	T (°C)	Median reaction times (MRT) and confidence limits in days*						
		First sets†	Pairs scored (No.)	Second sets‡	Pairs scored (No.)	Third party‡	Pairs scored (No.)	Third-party combination
COCO ⇌ MCAS	24	11.3 (10.5-12.2) (2.9)	47	7.2 (6.4-8.1) (2.2)	26			
COCO ⇌ MCAS	27	9.0 (8.3-9.8) (1.9)	24	3.8 (3.3-4.4) (0.9)	10	5.6 (4.9-6.4) (1.3)	11	COCO ⇌ KYC (naïve)
						6.8 (5.9-7.8) (1.5)	9	MCAS ⇌ KYC (naïve)
KYC ⇌ COCO	27	8.9 (7.0-11.4) (6.9)	30	4.2 (3.6-4.9) (1.3)	13	5.8 (4.6-7.3) (1.7)	6	KYC ⇌ MCAS (naïve)
						5.7 (4.3-7.5) (2.2)	7	COCO ⇌ MCAS (naïve)
KYC ⇌ MCAS	27	7.2 (6.4-8.2) (2.2)	21	4.0 (3.4-4.8) (1.2)	11	4.6 (4.1-5.2) (0.9)	12	KYC and MCAS to COCO (naïve) combined

\*These statistics were determined by the nomographic method of Litchfield (14); ranges in parentheses are 95 percent confidence limits and are equivalent to 2 standard errors of the median. Numbers in parentheses below each entry indicate 1 standard deviation of each MRT; this reflects the variation in individual pairs scored. †Each sequence of seven to nine pairs of first-set parabionts in each series were repetitively interclonal, that is, involved *Callyspongia* branches from the same two allogeneic colonies. ‡Interval between first-set and second-set or third-party parabioses was 15 to 16 days in the initial series at 24°C and 12 days in the remaining series at 27°C.

purple pigmentation throughout their soft tissues. The skeletal framework of fine horny fibers is brown in color and resistant to decomposition in seawater. Any soft tissue death quickly becomes visible by disappearance of purple-colored tissue from the skeletal framework. Clonal colony growth consists of long fingerlike projections from a common base attached to coral rock, and compatible intracolony graft connections are abundant in nature. Intercolony or allogeneic fusion of tissues, even where separate colonies are in close proximity, has yet to be observed. Tissue transplantation experiments were undertaken under controlled conditions in the running seawater system of the Hawaii Institute of Marine Biology. Intracolony (isogenic) and intercolony (allogeneic) combinations of branches of *Callyspongia* (each ~ 2 by 8 cm) were placed in close contact on separately tagged Plexiglas plates by a technique in which vinyl-covered, 18-gauge solid-core wire tie-downs were used (Fig. 1). Intracolony parabionts became firmly fused at all interfaces after 2 to 3 days and persisted thereafter in compatible confluence without exception (Fig. 1A). Allogeneic parabionts were extensively tested in three combinations from separate locations designated COCO (Coconut Island), MCAS (Marine Corps Air Station), and KYC (Kaneohe Yacht Club) (Table 1). Alloparabionts invariably failed to fuse despite intimate contact. After a latent period with no sign of antagonistic reaction in first-set grafting, interfacial soft tissue death developed beginning as early as 4 to 5 days in certain interclonal combinations. Graft interfaces were examined with the aid of a binocular head-

band magnifier. Allogeneic cytotoxicity was scored as definitive when soft tissue destruction extended 1.0 mm or more to either side of an interface (Fig. 1B). This "definitive" stage was reproducibly quantifiable by different observers. The sponges showed no tissue reaction to inert materials such as the Plexiglas plates or vinyl-covered wire employed.

During March, at a water temperature of 24°C, a median reaction time (MRT) of 11.3 days (10.5 to 12.2, within 95 percent confidence limits) was found for 47 first-set COCO ⇌ MCAS alloparabionts (the reversible arrows indicate the bidirectionality of the test parabioses). Similar reactivity occurred in other tests when grafts were orthotopically sutured in place. After 15 to 16 days when all initial parabionts had reacted to the convincing extent of 1 to 4 mm, second sets were established by placing the same pairs together at new interfaces at least 2 cm remote from the original contact zones. These repeat grafts consistently showed accelerated cytotoxic reactions with an MRT of 7.2 (6.4 to 8.1) days. A much larger series of first-set, second-set, and third-party parabioses were scored, in July and August 1978, at 27°C. At this higher temperature, both first- and second-set reactions in COCO ⇌ MCAS occurred significantly more rapidly than their counterparts at 24°C (Table 1), while the sharp distinction between primary and secondary MRT's was still evident. Third-party parabioses in lieu of second sets were performed between sensitized COCO or MCAS pieces and naïve KYC pieces soon after the completion of first-set reactions. Identical test conditions applied to second sets and third parties, all maintained in the

same large aquariums and scored concurrently. Third-party MRT's in both directions were similarly intermediate between first-set and second-set MRT's. These significant differences, evident from the nonoverlapping 95 percent confidence limits (Table 1), suggested both specificity in alloimmune memory and considerable sharing of H antigens among the three sources of *Callyspongia*.

This same experimental design was applied to the other possible combinations of allogeneic parabionts: KYC ⇌ COCO with third-party pairings to naïve MCAS and KYC ⇌ MCAS with third-party pairings to naïve COCO sponges. Each sequence of seven to nine pairs of first-set parabionts in the series reported here were interclonal replicates, involving *Callyspongia* fingers from the same two allogeneic colonies. This provided an additional control on the consistency of the observed reactions between the equivalent of separate inbred lines. The whole gamut of strong, moderate, and weak reactions occurred among the four different interclonal KYC ⇌ COCO combinations. The first-set MRT of 8.9 (7.0 to 11.4) days was accordingly intermediate between, and inclusive of, the other two interpopulation MRT's, but with a notably larger standard deviation. The substantially different strengths of histoincompatibility demonstrable as a function of the interclonal combination was also evident in the nonoverlapping first-set MRT's for COCO ⇌ MCAS versus KYC ⇌ MCAS at 27°C (Table 1). The interval between establishment of first-set and second-set or third-party parabioses was 12 days in all series at 27°C, but 15 to 16

days in the initial series at 24°C. Similarly, accelerated second-set reactions with MRT's of 3.8 to 4.2 days, indicative of at least short-term memory, were found in all three combinations at 27°C. Third-party MRT's reflected an array of accelerated and nonaccelerated reactions in all instances, except for the limited KYC or MCAS to naïve COCO group where most second-set and third-party reactions were of heightened intensity. Although early unilateral tissue damage to naïve sponges by sensitized sponges was

typical of accelerated third-party reactions as might be expected, concurrent bilateral cytotoxicity was often observed. Unilateral tissue death in the opposite direction was least frequent under these circumstances.

Some 200 different alloparabionts have proved incompatible without exception thus far, thereby revealing extensive polymorphism of cell-surface histocompatibility molecules within this species. Depending on the interclonal combination, reactions ranged from strong to

weak in *Callyspongia*. At this lower level of phylogeny, we were surprised to find (i) early and vigorous primary allograft rejection, (ii) conspicuous cytotoxic reactivity accompanying allogeneic incompatibility, and (iii) specific or selective alloimmunity with a memory component. Allogeneic incompatibilities reflected in consistent nonfusion reactions at 15°C were also demonstrated in two species of California sponges, *Axinella mexicana* and *Cyamon argon*, in controlled pilot experiments in California. We now conclude that exquisite immunorecognition of subtle differences among allogeneic markers is a characteristic of essentially all major groups of multicellular animals (13).

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7. We would identify three components as minimal criteria for immunological competence: (i) cytotoxic or antagonistic reaction following sensitization, (ii) selective or specific reactivity, and (iii) inducible memory or selectively altered reactivity on secondary contact. All three appear necessary because specificity and memory alone could apply to the central nervous system, while specificity and incompatibility alone include cell recognition reactions in protozoan and plant reproduction.
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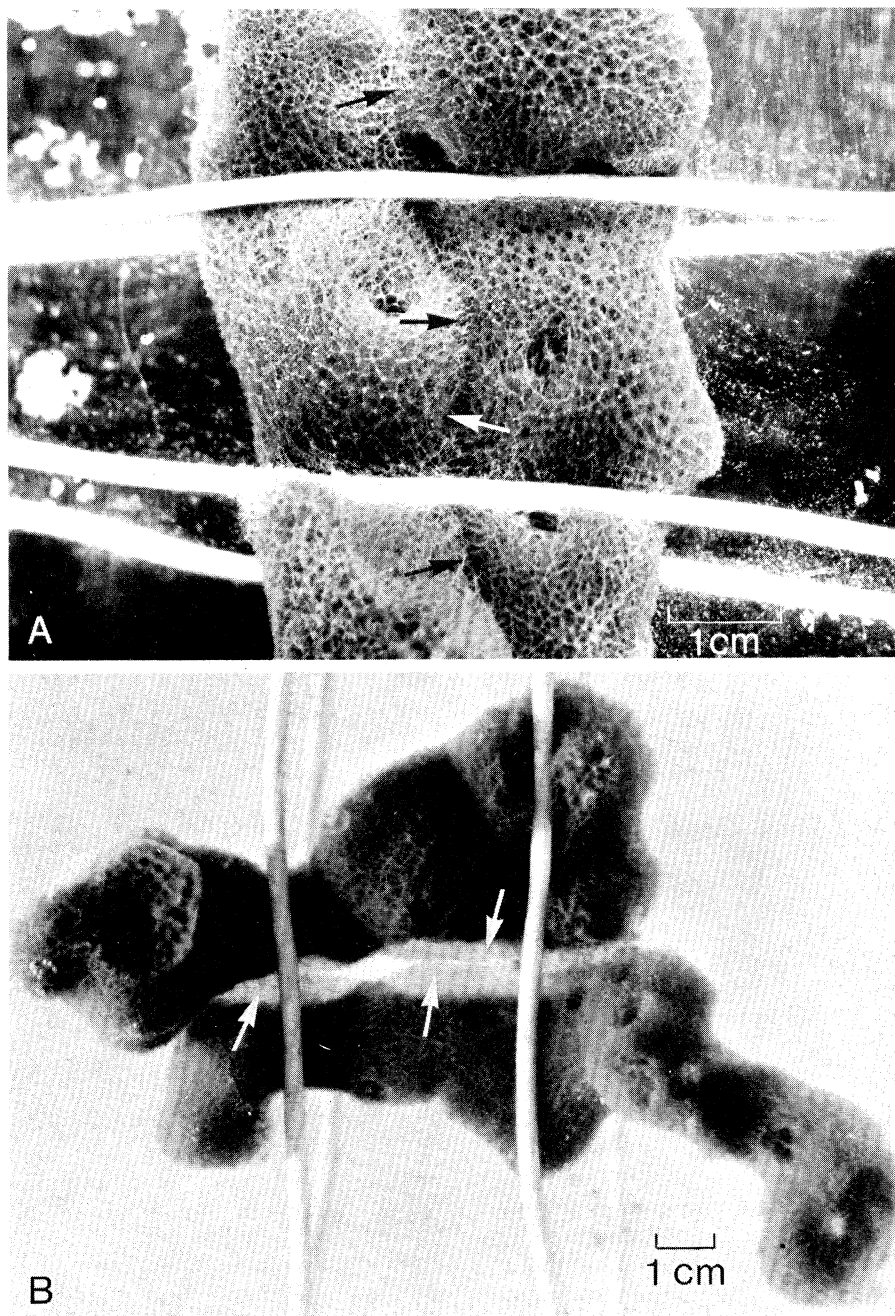


Fig. 1. Parabolic reactions between intact fingers of *Callyspongia* held together by vinyl-covered wire tie-downs on Plexiglas plates. (A) Compatible interfascial fusion (arrows) of syngeneic or isogeneic parabionts after 2 to 3 days in contact. (B) Incompatible bilateral cytotoxicity between allogeneic parabionts showing skeletal framework (arrows) after local soft tissue necrosis at 7 to 9 days.