0022-1767/84/1335-2351802.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 133, No. 5, November 1984
Printed in U.S.A.

ALLOIMMUNE MEMORY IS ABSENT IN *HYMENIACIDON SINAPIUM*, A MARINE SPONGE[1]

L. COURTNEY SMITH[2] AND WILLIAM H. HILDEMANN[3]

From the Immunogenetics Group, Department of Microbiology and Immunology, School of Medicine, University of California, Los Angeles, CA

The southern California sponge *Hymeniacidon sinapium* can recognize and reject transplanted allogeneic tissues. This species reveals a complex rejection response that involves cellular infiltration into the graft interface and a deposition at that site of a fibrous wall. The response then proceeds to a cytotoxic reaction in which the cells in the graft zone become necrotic and slough off, resulting in allogeneic tissue separation. The rate and intensity of this response varies with the genetic constitutions of the paired sponges and the temperature of the water. In experiments employing pre-sensitized second-set and unrelated third-party graftings, with changes in temperature, sensitization times, intervals before regrafting, and experimental sites, this species did not exhibit alloimmune memory (i.e., accelerated second-set rejection).

When the allorejection described here for *H. sinapium* is taken in conjunction with rejection responses reported for other sponge species, it is apparent that sponges have two major methods for responding to allogeneic contacts: barrier formation or cytotoxicity. The rejection method seems to correspond with the presence or absence of immune memory, and may, in part, be correlated with habitat and frequency of contacts with "non-self." The development of the rudimentary immune system in metazoans is discussed in relation to the rejection of naturally transplanted tissues in addition to challenges from pathogens.

Components of an adaptive immune system include: recognition of "non-self"; reaction to non-self by effector mechanisms such as cytotoxicity, phagocytosis, or encapsulation; and inducible memory, measurable as a heightened or accelerated response after re-contact with antigens to which the organism has previously been exposed (1). For many years, it was assumed that among metazoans, only vertebrates possessed immune systems with specificity and memory; this supposition is now changing. As increasing numbers of invertebrates are investigated, more and more species are found to exhibit the hallmarks of immunity (2). Although tissue transplantation is an artificial assay of general immune reactivity in vertebrates, it is a natural occurrence for many sedentary invertebrates and may be the most appropriate means for comparing immune responsiveness across the whole phylogenetic spectrum (1). Grafting experiments on several sponge species have shown that these animals readily exhibit the first two components of an immune system: the ability to recognize and to reject foreign tissue (3–13). However, the third component, memory, has been detected in only three of the six species of sponge tested for this characteristic (i.e., accelerated second-set rejection) (6–9, 12–15). Our present study with *Hymeniacidon sinapium*, a marine sponge found in the southern California marinas, reveals that this species recognizes and rejects all allografted tissue, but does not exhibit alloimmune memory.

MATERIALS AND METHODS

Animals. H. sinapium is abundant intertidally on rocks and other firm substratum along the southern California coastline. Experimental animals were collected from floating boat dock piers in several marinas, including the Channel Islands Marina, Marina del Rey, King Harbor, the Los Angeles Harbor, and the Long Beach Marina. Some animals were transported to the Pacific Biomarine Co. (Venice, CA), were held temporarily in a large recirculating sea water system (16°C), and were then transferred to the UCLA Biology Department aquarium room (a large recirculating sea water system, 12°C) or to a 520-liter Instant Ocean aquarium (variable temperature). After collection, other sponges were transported directly to the Instant Ocean aquarium at UCLA, or to the Marina del Rey Harbor Patrol dock where the *in situ* experiments were performed. The animals were placed in a box (2 ft x 3 ft x 1 ft) constructed of one-half-inch wire mesh, and were maintained just at the water surface with styrofoam floats.

Grafting techniques. The results from a pilot study investigating the genetic population structure of *H. sinapium* on a single boat pier in the Channel Islands Marina indicated a graft acceptance frequency of 62% when animals were collected from within 20 cm of each other (data not shown; see References 10, 16 and 17). Therefore, to avoid fusions between sponges and to concentrate on the rejection kinetics of this species, animals were paired either from separate sites within Marina del Rey or from separate marinas.

The grafting and scoring techniques used in this study were modified slightly from those described previously for several marine sponges (7, 11, 12). Direct contact of the surfaces of grafted individuals initiates rejection. However, because the cuticle of *H. sinapium* can prevent direct cellular contact, it was necessary to cut it away to permit direct cellular interactions to occur (see Reference 18). As documented for *Callyspongia diffusa*, a Hawaiian sponge, grafting at cut areas *vs* intact pinacoderm (surface cells) makes no difference in rejection rates (8).

Control autografts and experimental allografts were set up, securing pairs of contacted sponges with 6-lb nylon monofilament fishing line to individually labeled plastic splints (1 in. x 3 in.). Primary, secondary, and third-party allografts were performed with variations in pre-sensitization times, intervals between sensitization and regrafting, and temperature.

Graft interfaces were examined daily by using a dissecting micro-

Received for publication April 2, 1984.
Accepted for publication June 25, 1984.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This study was supported by National Institutes of Health Grants AI 15705 and AI 19470.
[2] Reprint requests and correspondence should be addressed to L. Courtney Smith, Department of Microbiology and Immunology, School of Medicine, UCLA, Los Angeles, CA 90024.
[3] Deceased; September 8, 1983.

scope or a head magnifier. Hyperplasia, tissue necrosis, and tissue separation were recorded as they appeared. End points were scored as day of tissue separation or 1 mm necrosis. The data were analyzed by the paired *t*-test (19) to compare the primary rejection time with the secondary or third-party rejection time for each genetic combination of sponges.

RESULTS

The response of H. sinapium to allografting. Autografts (intracolony grafts) fused compatibly at the interface in 1 or 2 days, and remained fused indefinitely. Allografts (intercolony grafts) were recognized and rejected as non-self tissue. Rejection could be macroscopically discerned as an infiltration of cells from each sponge into the graft zone. Hyperplastic buildup was soon followed by the appearance of a thin dark line defining the graft interface. Tissue necrosis at or near the graft interface eventually led to tissue separation and exposure of the spicular skeleton. Occasionally, the necrotic reaction left intact the dark line which appeared as a fibrous wall connected to the exposed spicules in the area. A similar structure has been observed in *Ephydatia fluviatilis*, a fresh-water sponge (20). On rare occasions, one sponge of a pair overgrew its own surface with new tissue. When this overgrowth tissue recontacted allogeneic tissue, the rejection response was reinitiated by another round of hyperplasia and necrosis.

As has been noted for other species, variation in the intensity of the rejections in *H. sinapium* were observed. It has been assumed that such variation was related to genetic differences between different pairs of sponges (8, 11, 13). "Weak" reactions, which began with minimal cell infiltration, usually lacked an obvious dark line at the interface and resulted in pairs that could be easily separated. Tissue necrosis began at the graft interface and proceeded through the hyperplastic regions back towards the adjacent normal tissues. Tissue separation was often 1 mm or less, and the sponges occasionally secreted cuticles at the new surface. "Vigorous" responses began with a massive infiltration of cells into the graft interface, which resulted in large areas of hyperplastic tissue that often protruded above the normal tissue surface and extended farther back from the interface. The dark line was usually quite prominent, revealing the interdigitation of hyperplastic tissues. With responses of this magnitude, pairs of animals could only be separated with damage. In these situations, the necrosis began at the graft interface, extended back through the hyperplastic tissue, and left an irregular separation gap of 1 to 2 mm. In cases of maximal hyperplastic responses, the necrosis began, not at the graft interface, but at the region between the hyperplastic and normal tissues in one or both animals. This eventually resulted in isolation or abandonment of the hyperplastic tissue with a disintegration of the entire graft zone, leaving a much larger separation gap (more than 2 mm). Eventually, the animals formed new pinacoderm over the normal tissue. In a few cases, sponges showed preferential growth (migration?) in regions distant from the area of contact. Such migration has been noted in allografts in *E. fluviatilis* (15, 20).

In most instances, the *H. sinapium* allorejection response was bilaterally cytotoxic; however, unilateral rejections occurred occasionally. In the more vigorous cases, one animal of a pair actively invaded the spicular skeleton exposed by the necrotic reaction in the other. This phenomenon has also been noted in *C. diffusa* (8).

The effects of temperature on allograft rejection. A pilot allorejection experiment (Table I, Expt. 1) was conducted in the UCLA marine aquarium facility. At 12°C, a temperature that is 2 to 7°C colder than normal for this species (the water temperatures recorded daily in Marina del Rey from mid-October to mid-December, 1983, ranged from 14 to 23°C), all 13 primary allografts showed evidence of cellular infiltration after 2.15 (± 1.57) days, which is significantly slower than hyperplastic responses of 1.21 (± 0.79) days ($n = 140$, $p < 0.05$) recorded in primary allorejections subsequently studied at higher (15 to 21°C) temperatures (data not shown). Four of the 13 pairs (31%) at 12°C failed to progress to necrosis after hyperplasia (compared to 100% of pairs that progressed to necrosis at higher temperatures). The remaining nine pairs gave necrotic responses to grafted tissues with an average onset time of 18.44 (± 8.07) days. This is significantly slower than responses at higher temperatures ($p < 0.01$). The effect of cold water on slowing the rejection process is clear from Figure 1, in which the mean necrotic onset time for primary allografts in each experiment is plotted relative to water temperature. Therefore, all subsequent experiments were carried out at higher temperatures.

The search for alloimmune memory. Once pilot studies (Table I, Expts. 1 and 2) revealed that *H. sinapium* could effectively reject allogeneic first-set grafts, the responses to secondary and third-party challenge grafts were investigated. Replicate pairs of pieces from the same animals were sensitized to each other for 2 to 3 days, separated for 7 days, and regrafted at sites remote from those used for priming. In addition, pre-sensitized sponges were paired to pieces of third-party animals. Unprimed sponges were grafted as primary controls. As noted in experiment 3 (Table I) at 21°C, no significant differences were seen between the primary and secondary or third-party grafts in the times for onset of necrosis or day of separation. This suggests that immune memory may be absent in this species.

Even though all priming grafts became hyperplastic within 1 day, it was thought that 2 to 3 days of presensitization was insufficient time to induce alloimmune memory. Priming for 4 days in *C. diffusa* ensures maximal sensitization in that species (8). Therefore, experiment 4 (Table I) was performed under the same aquarium conditions with new animals that were given a 7-day sensitization period and a 7-day interval before regrafting. Even though these allografts were scored for 1 mm of necrosis rather than tissue separation, no significant differences were evident between the primary, secondary, or third-party rejection times. Again, these data suggest a lack of memory.

Johnston *et al.* (21), investigating the effects of temperature on transplantation rejection for *C. diffusa*, found that, as the water temperature was increased towards the normal summer maximum, the rejection times and the differences between the primary and secondary rejections all decreased. Because the water temperature in the aquarium where the allografted *H. sinapium* were being maintained had been adjusted to near maximum for the intertidal waters of southern California, it was thought that the rejections were proceeding at maximal rates, thus obscuring the differences between primary

TABLE I

Allograft rejection in H. sinapium

Expt.	Sensitization Time in Days	Interval Between Primary and Secondary in Days	n	Median Reaction Time in Days[a] (±SD) Onset of Necrosis			Median Reaction Time in Days[a] (±SD) Tissue Separation			Temperature °C	Experimental Site[b]	Animal Collection Sites[c]
				primary	secondary	third-party	primary	secondary	third-party			
1	ND[d]	ND	9	18.44 (8.07)	ND	ND	ND	ND	ND	12	B	M
2	ND	ND	17	8.29 (2.94)	ND	ND	ND	ND	ND	17	A	M L K
3	2–3	7	8	11.13 (2.64)	9.56 (1.78)	9.00 (2.14)	17.19 (4.94)	17.74 (5.56)	15.87 (2.71)	21	A	M*
4	7	7	11	5.27 (1.29)	5.27 (1.06)	5.45 (1.03)	8.90[e] (2.64)	9.18[e] (2.95)	9.00[e] (1.79)	21	A	L M
5	4	8	4	9.00 (0.82)	9.88 (1.31)	12.25 (4.27)	20.5 (6.45)	20.00 (5.05)	21.75 (7.41)	15	A	M L K
6	7	14–15	10	6.70 (1.25)	7.05 (1.01)	7.30 (0.63)	16.85 (3.91)	17.40 (5.57)	22.47[f] (5.73)	18.5–23	I	M S
7	7	10	11	9.38 (3.75)	9.67 (3.33)	9.42 (2.46)	15.45 (6.58)	16.82 (5.55)	18.95 (6.27)	14–22	I	M S

[a] No statistical differences were found by using the paired *t*-test to compare the primary rejection time to that of the secondary or the third-party rejection time for each allografted pair of sponges. (At best, $p < 0.25$.)

[b] Experimental site: B = UCLA Biology Department recirculating sea water facility; A = Instant Ocean aquarium; I = *in situ* experiments in Marina del Rey.

[c] Animal collection sites: M = Marina del Rey; M* = Two different areas from within Marina del Rey; L = Los Angeles Harbor; K = King Harbor; S = Long Beach Marina.

[d] ND = Not done.

[e] The end point was scored at day of 1 mm necrosis rather than at day of separation.

[f] In this case, the third-party rejection time was significantly longer than that of the primary ($p < 0.025$).

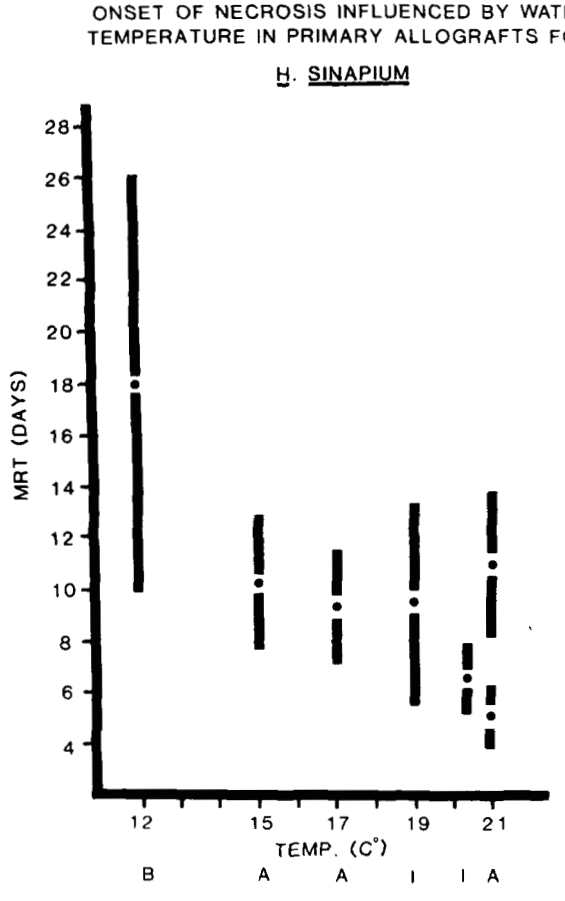

Figure 1. Data were taken from Table I. ● indicates mean, bars indicate ± SD. The necrotic onset is significantly slower ($p < 0.01$) at 12°C than at the higher temperatures. Experimental sites are indicated at the bottom; B = UCLA Biology Department marine aquarium facility, A = Instant Ocean aquarium, I = *in situ* experiments in Marina del Rey.

and secondary rejection times. Accordingly, experiment 5 (Table I) was performed at 15°C with newly collected animals sensitized for 4 days (8) before regrafting. Once again, no significant differences were noted.

To rule out the possibility that the sponges were reacting abnormally to grafted tissue due to improper nutrition, two additional experiments (6 and 7) were conducted with the sponges in their normal habitat where they had access to natural particulate food, normal light/dark cycles, fluctuations in the water temperature (14 to 23°C), and changes in salinity caused by fresh water runoff. For the first *in situ* experiment (Expt. 6), animals were collected from the Marina del Rey A basin and the Long Beach Marina and were transported to the Marina del Rey Harbor Patrol dock. After allowing the sponges to recover from collection and transport for at least 3 days, they were sensitized by parabiosis for 7 days. Duplicate secondary and third-party allografts were initiated 14 to 15 days after the end of the sensitization period, along with duplicate primary allografts. Third-party animals were collected from the Harbor Patrol dock. In experiment 7, animals from the Marina del Rey H basin and the Long Beach Marina were sensitized for 7 days, followed by a 10-day interval before regrafting. Except for water temperature, experiments 6 and 7 were essentially the same. As shown in Table I, no differences in rejection times were evident, again indicating that alloimmune memory is absent under the conditions employed in this series of experiments.

DISCUSSION

There are two major types of allograft rejection in sponges. Members of the Poriferan phylum invariably accept autografts. They can recognize non-self and effectively reject allogeneic tissue, apparently by two basically different mechanisms (6). The first involves "wall formation" (perhaps equivalent to an encapsulation attempt on an object too large to cover), in which the allografted sponges (Table II, species 1 to 4) secrete collagenous or cuticular barriers at the graft interface which effect tissue separation. This type of allograft response is not associated with cellular infiltration, cellular exchange, cytotoxicity, or necrosis along the separating barriers. The second type of allograft rejection does not involve

TABLE II

The method of graft rejection in sponges correlates with immune memory

Species	Collagen Barrier	Cellular Infiltrate	Cytotoxicity	Immune Memory	Reference
1. *Verongia longissima*	Yes	No	No	No?[a]	10, 17
2. *Verongia thiona*	Yes	No	No	No?	L. C. Smith, unpublished
3. *E. fluviatilis*	Yes	No	No	No	6, 16
4. *Axinella verrucosa*	Yes[b]	Yes	?[c]	No	13
5. *Axinella polypoides*	No	Yes	Yes	No	6
6. *H. sinapium*	Yes[d]	Yes	Yes	No	This paper
7. *H. perleve*	No	Yes	Yes	Yes	9
8. *C. diffusa*	No	Yes[e]	Yes	Yes	7, 8, 14, 18
9. *T. violacea*	No	Yes[e]	Yes	Yes	12
10. *Xestospongia exigua*	No	Yes[e]	Yes	Yes?[a]	11
11. *Suberites domuncula*	No	Yes[f]	?[b]	Yes?	4
12. *Tethya lyncurium*	No	Yes[e]	No[g]	?[h]	3, 4
13. *Ectyoplasia ferox*	No	No	No[i]	?	22
14. *Iotrochota birotulata*	No	No	No[j]	?	17
15. *Leucosolenia losangelensis*	No	No	No[j]	?	L. C. Smith, unpublished

[a] Predictions on the presence (Yes?) or absence (No?) of immune memory are followed with a question mark.

[b] *A. verrucosa* responds to different allografted tissues with variable degrees of wall formation. The mild allorejection results in an increase in the normal collagen network, which does not appear to act as a barrier; more severe reactions involve true barrier formation.

[c] Information is not available on the involvement of cytotoxicity in the allorejections of *A. verrucosa* and *S. domuncula*.

[d] The material deposited at the *H. sinapium* graft interface has not been characterized. Its porosity is not known.

[e] The cellular infiltration leads to the formation of tissue bridges before proceeding to cytotoxic and necrotic reactions.

[f] *S. domuncula* shows extensive cellular infiltrations from both graft and host tissues into the graft zone and appears similar to other cytotoxic responses. Unfortunately, the final method of tissue separation was not described.

[g] *T. lyncurium* extrudes small orthotopically fitted allografts from the host sponge, a process which appears similar to asexual budding in this species.

[h] Insufficient information for predictions on the memory response are indicated with a question mark.

[i] *E. ferox* was reported to show a "decellularized" region at the graft zone because cytotoxicity and necrosis were not observed (see text for discussion).

[j] *I. birotulata* and *L. losangelensis* show no overt reaction to allografted tissue.

barrier formation. Instead, the sponges (Table II, species 5 and 7 to 11) respond to direct and continued surface contact with cellular infiltration into the graft zone, which leads to cytotoxic reactions, tissue necrosis, disintegration, and allograft separation.

Alloresponses of certain species do not fit into either of these two allograft rejection categories (Table II, species 6 and 12 to 15). As presented, *H. sinapium* (Table II, species 6) exhibits both barrier formation and cytotoxicity, whereas other sponges (Table II, species 14 and 15) show no overt responses of either type (although histologic investigations might reveal subtle reactions not obvious on macroscopic inspection; *cf* 23). In another species (Table II, species 12), host sponges slowly extrude small orthotopically fitted allografts after cellular infiltration into the graft interface. Finally, speculations on the "decellularized" zone noted in the reported allorejections in a Caribbean sponge (Table II, species 13) suggested cellular migration away from the graft site, because neither barrier formation nor cellular infiltration and cytotoxicity were observed (22). However, because this study was done *in situ* and transplants were scored through a diving mask on days 5, 7, and 9 after grafting, a hyperplastic and cytotoxic response leading to a vigorous necrotic reaction may have been missed. As a case in point, *Toxadocia violacea* can show complete rejections in 2.5 days or less in warm Hawaiian waters (26 to 28°C) which are similar to temperatures in the Caribbean (12).

Only one-half of the sponge species that have been examined exhibit immune memory. Convincing demonstrations that Poriferans can recognize and reject artificially introduced foreign tissue implies that these animals can respond to natural contact with allogeneic or xenogeneic tissue. Sequential mapping of areas inhabited by encrusting sponges in space-limiting habitats indicates how sponges can spread, contact neighbors, regress, break up, and recoalesce over time (24, 25). Growth rates compared to the amount of cytotoxic damage inflicted by allogeneic contact in *C. diffusa* indicate that this species could regrow into contact with a neighbor before the specific short-term memory expires (14). This finding suggests that an adaptive immune system could aid in spatial competition in crowded habitats.

The existence of immunologic memory has been addressed for the few sponge species listed within the box in Table II. Memory, as measured by an accelerated second-set rejection of allogeneic tissue, is present in a few species (Table II, species 7 to 9). It is noteworthy that these three species mount cytotoxic responses to allogeneic tissue; they *do not* secrete barriers. Three of the four species reported to lack memory (Table II, species 3, 4, and 6) *do* produce barriers to effect tissue separation. This trend may allow predictions of the secondary responses (i.e., presence or absence of memory) based on the mechanism that each species employs for primary rejection. (*A. polypoides* (Table II, species 5) is an exception to this proposed theory.) Because effective barrier formation (and perhaps cuticular secretion) would minimize repeated contacts with neighbors, sponges showing this response might lack alloimmune memory. Alternatively, sponges exhibiting cytotoxic reactions without barrier formation could have repeated contacts with neighbors, and might be expected to display memory. (Memory predictions for the four species listed at the bottom of Table II (species 12 to 15) are not possible because descriptions of their graft rejections are either incomplete or do not fall into the two basic categories of rejection.)

Why should sponges show different types of allograft rejection, only one of which includes memory? An explanation based on taxonomic affinities is untenable because the two *Hymeniacidon* species fall into different rejection categories (Table II, species 6 and 7). An examination of their respective morphologies, growth rates, and habitats may provide potential correlations with the

presence or absence of immune memory. *Hymeniacidon perleve*, an encrusting species that spreads over rocks in the Taynish Channel in Scotland, responds cytotoxically on contacting its allogeneic neighbors and forms "buffer" spaces or "non-coalescent interfaces" between them (26). *H. sinapium*, with an amorphous morphology, does not cover large areas of rocks and piers in the California marinas, nor does it often contact its neighbors. Although there is no growth rate information available for these two species, a high growth rate within a crowded habitat would result in numerous contacts between individuals of *H. perleve* and neighboring sponges (see plate 1, Reference 26) over time periods perhaps shorter than that of short-term immune memory demonstrated for this species (9). Alternatively, a slow growth rate in an uncrowded habitat would not present *H. sinapium* with the same challenges. Immune memory would carry an adaptive advantage in *H. perleve*, whereas its absence in *H. sinapium*, where few or no allogeneic contacts occur, would be adaptively neutral. As a corollary to the above speculation on small or encrusting sponges, colonies with long-branching morphologies growing in close proximity might be brought into repeated contact by water currents. This phenomenon has been observed for several large colonies of *C. diffusa* (in which immune memory is present) growing near Coconut Island in Kaneohe Bay, Hawaii (W. H. Hildemann, unpublished observation).

The major challenges to the sponge immune system, however, may be only partially correlated with the space-limiting parameter of the habitat. An alternative model is needed for *T. violacea*, which is rarely found in close proximity to other sponges, and which has a globular rather than a ramose morphology. It has been suggested that the discriminating and vigorously cytotoxic immune system with memory exhibited by this species acts primarily in defense against pathogens rather than in defense against encroaching neighbors (12). Essentially, those sponges living in overcrowded conditions would be continually contacted by non-self tissues from other sedentary metazoans (including sponges) and by potentially pathogenic microbial organisms, whereas with other sponges, inhabiting areas where sedentary organisms are widely separated, the major non-self challenges would be presented primarily by microbes (14). Maintenance of self-integrity through rejection of naturally transplanted tissues must have employed effector mechanisms that could also act on infectious agents. Conversely, defense against single-celled organisms could also be effective against other metazoans in defense of self-integrity and avoidance of chimerism. During the development of the rudimentary immune system in metazoans, the ability to reject transplanted tissue may have been of equal importance as that for defense against pathogens, and this ability of the immune system to recognize and reject allogeneic and xenogeneic non-self appears to have been either maintained and passed on or repeatedly rediscovered in many other phyla during metazoan evolution.

Acknowledgments. The authors are grateful to Dr. C. H. Bigger for assistance and suggestions offered during the course of the investigation; to Dr. E. L. Cooper for the use of his Instant Ocean aquarium; to Mr. D. Doyle for his laboratory and photographic assistance; to Drs. B. Bonavida, E. L. Cooper, L. Muscatine, J. Bramhall, V. L. Scofield, N. Cohen, an anonymous reviewer, and Ms. A. K. Taylor for constructive criticism on the manuscript; and to the Marina del Rey Harbor Patrol officers for their cheerful cooperation.

REFERENCES

1. **Hildemann, W. H., C. H. Bigger, P. L. Jokiel, and I. S. Johnston.** 1980. Characteristics of immune memory in invertebrates. In *Phylogeny of Immunological Memory.* Edited by M. J. Manning. Elsevier/North-Holland Biomedical Press, Amsterdam. P. 9.
2. **Manning, M. J., ed.** 1980. *Phylogeny of Immunological Memory.* Elsevier/North-Holland Biomedical Press, Amsterdam.
3. **Paris, M. J.** 1957. Greffes homoplastiques et bourgeonnement expérimental chez *Tethya lyncurium* (Lamark). *CR Acad. Sci. 245:578.*
4. **Paris, M. J.** 1961. Greffes et sérologie chez les éponges siliceuses. *Vie et Milieu Suppl. 11.*
5. **Van de Vyver, G.** 1970. La non confluence intraspecifique chez les spongiaires et la notion d'individu. *Ann. Emb. Morph. 3:251.*
6. **Van de Vyver, G.** 1980. Second set allograft rejection in two sponge species and the problem of an alloimmune memory. In *Phylogeny of Immunological Memory.* Edited by M. J. Manning, Elsevier/North-Holland Biomedical Press, Amsterdam. P. 15.
7. **Hildemann, W. H., I. S. Johnston, and P. L. Jokiel.** 1979. Immunocompetence in the lowest metazoan phylum: transplantation immunity in sponges. *Science 204:420.*
8. **Hildemann, W. H., C. H. Bigger, I. S. Johnston, and P. L. Jokiel.** 1980. Characteristics of transplantation immunity in the sponge *Callyspongia diffusa. Transplantation 30:362.*
9. **Evans, C. W., J. Kerr, and A. S. G. Curtis.** 1980. Graft rejection and immune memory in marine sponges. In *Phylogeny of Immunological Memory.* Edited by M. J. Manning. Elsevier/North-Holland Biomedical Press, Amsterdam. P. 27.
10. **Kaye, H., and T. Ortiz.** 1981. Strain specificity in a tropical marine sponge. *Marine Biol. 63:165.*
11. **Hildemann, W. H., and D. S. Linthicum.** 1981. Transplantation immunity in the Palaun sponge *Xestospongia exigua. Transplantation 32:77.*
12. **Bigger, C. H., P. L. Jokiel, and W. H. Hildemann.** 1983. Cytotoxic transplantation immunity in the sponge *Toxadocia violacea. Transplantation 35:239.*
13. **Buscema, M., and G. Van de Vyver.** 1983. Variability of allograft rejection processes in *Axinella verrucosa. Dev. Comp. Immunol. 7:613.*
14. **Bigger, C. H., P. L. Jokiel, W. H. Hildemann, and I. S. Johnston.** 1982. Characterization of alloimmune memory in a sponge. *J. Immunol. 129:1570.*
15. **Van de Vyver, G.** 1983. Absence of alloimmune memory in the freshwater sponge *Ephydatia fluviatilis. Dev. Comp. Immunol. 7:609.*
16. **Jokiel, P. L., W. H. Hildemann, and C. H. Bigger.** 1982. Frequency of intercolony graft acceptance or rejection as a measure of population structure in the sponge *Callyspongia diffusa. Marine Biol. 71:135.*
17. **Neigel, J. E., and J. C. Avise.** 1983. Histocompatibility bioassays of population structure in marine sponges. *J. Hered. 74:134.*
18. **Bigger, C. H., W. H. Hildemann, P. L. Jokiel, and I. S. Johnston.** 1981. Afferent sensitization and efferent cytotoxicity in allogeneic tissue responses of the marine sponge *Callyspongia diffusa. Transplantation 31:461.*
19. **Brown, B. W., and M. Hollander.** 1977. *Statistics, a Biomedical Introduction.* John Wiley and Sons, Inc., New York.
20. **Van de Vyver, G., and L. de Vos.** 1979. Structure of a non-merging front between two fresh water sponges *Ephydatia fluviatilis* belonging to different strains. In *Colloques du CNRS No. 291. Biology des Spongiares.* Edited by C. Levy and N. Boury-Esnault. Editions du CNRS, Paris. P. 233.
21. **Johnston, I. S., P. L. Jokiel, C. H. Bigger, and W. H. Hildemann.** 1981. The influence of temperature on the kinetics of allograft reactions in a tropical sponge and a reef coral. *Bio. Bull. 160:280.*
22. **Curtis, A. S. G., J. Kerr, and N. Knowlton.** 1982. Graft rejection in sponges. *Transplantation 33:127.*
23. **Vanadarajan, J., and R. D. Karp.** 1983. Histological versus morphological assessment of graft rejection in invertebrates. *Transplantation 35:629.*
24. **Sara, M.** 1970. Competition and cooperation in sponge populations. *Symp. Zool. Soc. Lond. 25:273.*
25. **Johnson, M. F.** 1979. Recruitment, growth, mortality and seasonal variations in the calcareous sponges *Clathrina coriacea* (Montagu) and *Clathrina blanca* (Miklucho-Maclay) from Santa Catalina Island, California. In *Colloques Int. du CNRS No. 291. Biology des Spongiares.* Edited by C. Levy and N. Boury-Esnault. Editions du CNRS, Paris. P. 235.
26. **Curtis, A. S. G.** 1979. Individuality and graft rejection in sponges or a cellular basis for individuality in sponges. In *Systematics Association Special Volume No. 11. Biology and Systematics of Colonial Organisms.* Edited by G. Larwood and B. R. Rosen. Academic Press, New York. P. 39.