INVERTEBRATE HISTORECOGNITION

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Cover illustration

A competitive interaction between two colonies of the athecate hydroid *Hydractinia echinata*. Colonies are composed of feeding polyps, as well as two kinds of gastrovascular tissue, mat and stolons. Modified stolons laden with nematocysts are generally induced when two incompatible strains come into combat. This zone of combat, with a large mass of hyperplastic stolons, lies between the central regions of the two colonies.

Drawn by Monica Turner from a photograph by R. K. Grosberg

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THE ROLE OF MESOHYL CELLS IN SPONGE ALLOGRAFT REJECTIONS

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INTRODUCTION

H. V. Wilson, in 1907, attempted and failed to produce chimeric sponges from xenogeneic (different species) mixtures of dissociated sponge cells. His failed experiment, however, initiated an interest in cellular recognition and interaction that has continued to the present. Studies on cell communication have expanded to involve many types of cells from organisms of all kinds, while interest in sponge cell recognition and aggregation has endured. More recent work has elucidated the reactions of intact sponges to naturally and artificially transplanted allogeneic (same species) tissues, and some initial information on cell function that is important in these reactions has been reported. In this chapter, I will review the current knowledge about the involvement of sponge cells in allograft rejections as studied in whole, intact sponges, and about the relationships between cell aggregation and allogeneic reactions as studied *in vitro,* using allogeneic mixtures of dissociated cells. Analyses and discussions of various sponge species will be mainly limited to data that include identification of the cells involved.

BACKGROUND: NORMAL SPONGE ANATOMY

Before proceeding with the discussion of allograft rejections in various sponge species, a brief description of the normal leuconoid anatomy and path of the water current in the demospongia should allow useful comparisons to the altered structure of rejecting tissues (Figure 1; for general reviews on sponge anatomy, see Bergquist 1978; Simpson 1984). The skeleton of a sponge supports the cellular mass and is made of spicules. These spicules are held in place by cells or are "cemented" together by the deposition of spongin or collagen. By gross observation, the sponge body can be subdivided into two general areas: the ectosome and the choanosome. The ectosome encompasses the peripheral regions of the sponge and shows varying thicknesses and development depending on the species. The outermost layer of tissue makes up the exopinacoderm, which is composed of thin, flattened exopinacocytes,

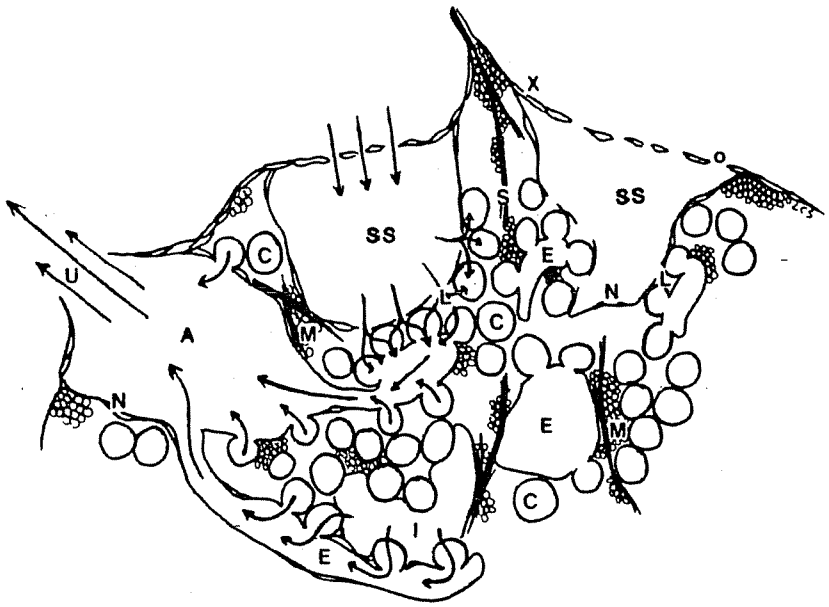

Figure 1. The pathway of water flow through a demosponge. Water (arrows) enters through the incurrent pores or ostia (O) in the exopinacoderm (X). From the ostia, which lead into the subdermal spaces (SS) and then to the endopinacocyte-lined (N) incurrent canals (I), the water passes into the lacunar spaces (L) and then between the cell bodies of the choanocytes that make up the choanocyte chambers (C). After passing through the chamber, the water enters the excurrent canal (E), several of which join to form the atrium (A) located below the excurrent pore or osculum (U). M, mesohyl S, spicular skeleton. (from Smith and Hildemann 1988)

and encloses the subdermal spaces. The exopinacoderm is perforated with many openings, or ostia, through which the water passes into the subdermal spaces. The choanosome, which encompasses the inner regions of the sponge, includes endopinacocyte-lined aquiferous canals, choanocyte chambers (the water pump and food filter), and mesohyl. Each choanocyte in the choanocyte chambers is equipped with a flagellum that beats to create the water current, and a ring of microvilli (the collar) that function in food filtration (Johnston and Hildemann 1982; Langenbruch 1983). The mesohyl is the true internal region of the sponge. It is covered on all sides by either endopinacocytes or choanocytes, does not contact the sea water, and is composed of a variety of cell types that vary somewhat from one species to another.

The aquiferous canal system in a leuconoid demosponge includes incurrent and excurrent canals, at the junction of which are found the choanocyte chambers (Figure 1). Water flows through the ostia into the subdermal spaces in the ectosome, into the incurrent canals that branch, decrease in size, and lead to the choanocyte chambers in the choanosome. After passing through the chamber, water leaves the sponge through the excurrent canal system where the canals combine to form those of increasing size

until reaching the atrium. This is the large space located below the osculum (the excurrent pore) in the ectosome through which the water passes to exit the sponge.

The mesohyl is composed of several cell types, only a few of which are germane to our discussion. These are the archeocytes, collencytes, spherulous cells, and in some cases, gray cells. Archeocytes are found in all demospongia and can be most easily identified by their large nucleolate nuclei even though they are morphologically heterogeneous, and contain varying numbers and sizes of inclusions. Archeocytes are amoeboid, phagocytic and are generally considered to be totipotent stem cells capable of reconstituting any cell type within the sponge (Borojevic 1966; De Sutter and Van de Vyver 1977; Buscema and Van de Vyver 1984c; Smith and Hildemann 1988). Collencytes are collagen secretory cells, and are normally involved in producing the non-spicular elements of the support structure (Garrone 1969; Connes *et al.* 1972). The gray cells, which contain large amounts of glycogen and may act as nutrient reserves (Boury-Esnault 1977), have also been reported to secrete collagen (Garrone 1974; Diaz 1979). Spherulous cells contain large spherical inclusions, the contents of which have been speculated to be involved in non-specific defenses based on their antibiotic actitivy (Bretting *et al.* 1983; Thompson *et al.* 1983). These cells have been noted in groups near subdermal spaces, areas of incurrent water flow where pathogens, along with food, would enter the sponge (Bretting and Königsmann 1980; Bretting *et al.* 1983; Smith and Hildemann 1988).

TRANSPLANTATION STUDIES

The outermost layer of tissue in sponges consists of live cells, so the consequence of growth and contact with other animals is natural tissue transplantation. Under conditions of limited space, natural allogeneic and xenogeneic contacts with other metazoans would be common. Paris (1961) was the first to perform controlled allografting in two marine sponges, *Tethya lyncurium* and *Suberites domuncula*. Van de Vyver (1970) analyzed the phenomenon of tissue fusion or non-fusion in parabioses from newly hatched gemmules of *Ephydatia fluviatilis*, a fresh water sponge. These first observations of allograft rejections in sponges initiated the study of sponge histocompatibility, which soon expanded to several additional species. Although it had been previously assumed from the xenogeneic cell aggregation studies that sponges were only capable of xenogeneic recognition, investigations on many species since then have established beyond doubt that sponges recognize and reject allogeneic tissues.

Most sponges react to allografts by one of two mechanisms (Table 1). These are (a) the construction of a barrier between the grafted tissues, or (b) a cytotoxic reaction at the graft interface that destroys the contacting tissues. Both methods effectively separate allogeneic cells. In addition, there are other means by which a few species reject tissues (Table 1, species 10, 18, 19). *Hymeniacidon sinapium* does both; it builds a barrier and reacts cytotoxically (Smith and Hildemann 1984). *Tethya lyncurium* shows neither barrier formation nor cytotoxicity, but does show measohyl cell infiltration of the graft side and extrudes small inserted grafts as if they were asexual buds (Table 1-Paris 1961). *Iotrochota birotulata* shows no overt reaction to allografted tissues, although it should be noted that allografts do not fuse (Neigel and Avise 1983). *Polymastia mamillaris* also shows minimal reaction to allografts by gross

Table 1. Two methods of allograft rejection in sponges (barrier formation and cytotoxicity) and their relationships to cellular infiltration and immune memory.

Species	Barrier Formation	Cyto-toxicity	Cellular Infiltrate	Immune Memory	Sources[i]
1. *Aplysina cauliformis*	+[a]	–	–[b]	ND[c](–)	1, 2
2. *Aplysina thiona*	+	–	–	ND(–)	3
3. *Aplysina fistularis*	+	–	?[d]	ND(–)	4
4. *Axinella damicornis*	+/–[e]	–	+	ND(–)	5
5. *Polymastia robusta*	+	?	+	ND(–)	6
6. *Polymastia mamillaris*	+/–	?	+	ND(–)	6
7. *Ephydatia fluviatilis*	+	–	–	–	7, 8
8. *Axinella verrucosa*[f]	+	?	+	–	5
9. *Microciona prolifera*	+	–	+	–	9, 10, 11
10. *Hymeniacidon sinapium*	+	+	+	–	12
11. *Axinella polypoides*	+/–	+	+	–	7, 13
12. *Hymeniacidon perleve*	–	+	+	+	14
13. *Callyspongia diffusa*	–	+	+	+	15, 16, 17
14. *Toxadocia violacea*	–	+	+	+	18
15. *Xestospongia exigua*	–	+	+	ND(+)	19
16. *Suberites domuncula*	–	?	+	ND(+)	20
17. *Haliclona aquaeductus*	–	+	?	ND(+)	21
18. *Tethya lyncurium*[g]	–	–	+	ND	22
19. *Iotrochota birotulata*[h]	–	–	–	ND	2

[a] + = present (+) = predicted to be present
[b] – = absent (-) = predicted to be absent
[c] ND = Not determined
[d] ? = unknown
[e] +/- = collagen deposition that is not a true barrier
[f] *A. verrusoca* has several types of responses. The information included in this table is that for the chronic rejection.
[g] *T. lyncurium* extrudes small fitted grafts much like asexual budding.
[h] *I. birotulata* shows no response to allogeneic contact.
[i] Sources are; [1]Kaye and Ortiz 1981; [2]Neigel and Avise 1983; [3]L. C. Smith unpublished; [4] Neigel and Schmahl 1984; [5]Buscema and Van de Vyver 1984b; [6]Van de Vyver and Barbieux 1983; [7, 8]Van de Vyver 1980, 1983; [9]Simpson, 1973; [10]Zea *et al.* 1986; [11]T. Humphreys, personal communication; [12]Smith and Hildemann, 1984; [13]Buscema and Van de Vyver 1984a; [14] Evans *et al.* 1980; [15, 16]Hildemann *et al.* 1979; 1980; [17]Bigger *et al.*1982; [18]Bigger *et al.* 1983; [19]Hildemann and Linthicum 1981; [20]Paris 1961; [21]C. H. Bigger, personal communication; [22]Paris 1961.

observation, but by histological analysis, cellular migration and collagen deposition could be identified (Van de Vyver and Barbieux 1983). Histological analysis will probably become very important for those species that show subtle rejections.

Before proceeding to a discussion of the cytological sequence of events that occur during allograft rejection, it would be helpful to describe briefly the events that occur during autograft fusion. It should be noted that for all sponges studied thus far, fusion of tissues to self proceeds in an identical manner, even though they show different methods of allogeneic tissue separation.

The exopinacoderms, which are the outer-most tissue layers, are the first to come into contact. They fuse together and then break up, bringing together the ectosomal areas and connecting the superficial regions of the aquiferous canal systems. This is followed by a removal or filling in of the large subdermal spaces, a rearrangement that joins the choanosomes and mesohyl, and completely fuses the aquiferous systems. The tissues merge so completely that it eventually becomes impossible to discern the original plane of contact. This process can occur quite swiftly in *Callyspongia diffusa*, being completed in one to three days (Hildemann *et al.* 1979; Smith and Hildemann 1986a), while in *Axinella polypoides,* the initial ectosomal fusion step can take several days with the entire process requiring up to a month to complete (Buscema and Van de Vyver 1984a).

Exopinacoderm contact in an allograft rejection, which appears similar to the initial contact in an autograft, soon proceeds to the obliteration of the pinacoderms by infiltrating mesohyl cells that quickly begin to accumulate at the graft interface ("cellular infiltrate" in Table 1). In most cases, streams or tracts of mesohyl cells have been noted, which is apparently one method by which large numbers of cells can be transported swiftly to the graft zone (Table 2). These ephemeral structures can be seen in normal mesohyl tissues but they are found in greater numbers during rejections and are oriented towards areas of allogeneic contact in *C. diffusa* (Smith and Hildemann 1986a). Yet, mesohyl cell tracts are apparently not absolutely necessary for mesohyl cell accumulations at the graft interface. They were not reported in studies of *Axinella verrucosa, Axinella damicornis* (Buscema and Van de Vyver 1984b), *Hymeniacidon perleve* (Evans *et al.* 1980), or *Microciona prolifera* (Zea *et al.* 1986; T. Humphreys, personal communication), although the authors did not state that they were absent. But not all allograft pairings in *C. diffusa* show mesohyl tracts, even though all allograftings show mesohyl cell accumulations at the interface (Smith and Hildemann 1986a). This suggests that general cellular migration through the mesohyl must be an additional method of cell accumulation.

Infiltrating mesohyl cells initiate major changes in the anatomy of the ectosome. The subdermal spaces (if pronounced in a species) disappear, and the choanocyte chambers in the area are removed or pushed aside by the infiltrating cells. These events are quite dramatic in micrographs of rejection zones in *Polymastia robusta, Polymastia mamillaris* (Van de Vyver and Barbieux 1983), *A. verrucosa* (Buscema and Van de Vyver 1984b) and *C. diffusa* (Johnston and Hildemann 1983; Smith and Hildemann 1986a,b). In addition, careful analysis of the accumulated mesohyl cells at the graft interface shows that the cells line up in "fronts" on either side of the plane of contact in several species (Van de Vyver and Barbieux 1983; Buscema and Van de Vyver 1984b; Smith and Hildemann 1986a). Even though these fronts of cells are not clearly seen in *E. fluviatilis*, microcinematography was used to show that no cell crossed the graft boundary before the barrier was laid down (Van de Vyver and De

Table 2. The cell type in the rejection zone correlates to the method of allograft rejection in sponges.

Species	Major Cell Type	Mesohyl Tracts	Collagen Barrier	Cyto-toxicity	Sources[e]
1. *Hymeniacidon perleve*	archeocytes	?[a]	–[b]	+[c]	1
2. *Callyspongia diffusa*	archeocytes	+	–	+	2, 3, 4
3. *Axinella polypoides*	archeocytes	+	+/–[d]	+	5
4. *Suberites domuncula*	archeocytes	+	–	?	6
5. *Axinella damicornis*	collencytes, spherulous cells		+	–	7
6. *Axinella verrucosa*					7
a. non-fusion	none	–	–	–	
b. fusion	collencytes, archeocytes	–	–	–	
c. chronic rejection	collencytes	?	+	–	
7. *Polymastia mamillaris*	collencytes archeocytes gray cells	+	+	?	8
8. *Polymastia robusta*	collencytes, archeocytes	+	+	–	8
9. *Microciona prolifera*	gray cells	?	+	–	9, 10

[a] ? = unknown
[b] – = absent
[c] + = present
[d] +/– = collagen deposition that is not a true barrier
[e] Sources are; [1]Evans *et al.* 1980; [2]Johnston and Hildemann 1983; [3, 4]Smith and Hildemann 1986a,b; [5]Buscema and Van de Vyver 1984a; [6]Paris 1961; [7]Buscema and Van de Vyver 1984b; [8]Van de Vyver and Barbieux 1983; [9] Zea and Humphreys 1985; [10]Zea *et al.* 1986

Vos 1979). Immunohistological studies using a monoclonal antibody directed to an individual *C. diffusa* marker, showed that the cells at the allograft interface did not mix at any time during rejection and were not mixed after tissue separation had occurred (Figure 2; Smith and Hildemann 1986b).

After the mesohyl cells have accumulated, some species secrete a collagenous barrier from both sides of the interface (Table 1, species 1-11). This barrier can range in thickness from a 150-μm collagen sheet (Van de Vvyer and Barbieux 1983; Buscema and Van de Vvyer 1984b) to a loose collagen network that does not appear to act as a barrier (Buscema and Van de Vyver 1984a). Other species exhibit a cytotoxic reaction after the mesohyl cells have accumulated, resulting in the destruction of the tissues at the interface, the exposure of the underlying spicules, and the separation of the grafted pair (Table 1, species 10-15, 17). The extent of tissue destruction can range from the archeocytes just at the zone of contact (Buscema and Van de Vyver 1984a) to the death of the entire sponge (Hildemann *et al.* 1980).

The cellular infiltrate that accumulates at the graft interface may include archeocytes, collencytes, spherulous cells and in a few cases, gray cells. Table 2 lists those species for which histological and/or ultrastructural information on allograft rejections is available. Those species that show cytotoxicity have archeocytes that interact at the graft interface (Table 2, species 1-4), while those species that erect barriers in response to allogeneic contact have collencytes (Table 2, species 5-8) and in one species, gray cells (Table 2, species 9) that line up in fronts at the graft interface. This suggests that when collencytes, or other collagen secreting cells such as gray cells, are involved with the rejection process, a collagen barrier is secreted between the fronts of cells. When archeocytes are involved and collencytes are absent, cytotoxic responses to allogeneic contact are displayed.

Alloimmune memory and recognition specificity in sponges have been demonstrated by an increase in the speed and magnitude of the responses in secondary rejections as compared to primary and third-party allografts. These types of experiments have been done on several species, and only some of them show immune memory (Smith and Hildemann 1984). The total duration of immune memory has only been analyzed in *C. diffusa,* and lasts for three to four weeks (Bigger *et al.* 1982). Memory is present in *Toxadocia violacea* at 19 days (Bigger *et al.* 1983) and in *H. perleve* at one week (Evans *et al.* 1980) but the total duration has not been determined in these species. Immune memory seems to correlate to the type of rejection observed in a sponge; barrier forming sponges do not secrete the barriers faster upon rechallenge (Table 1, species 7-10), but cytotoxically reacting sponges show a general increase in speed and magnitude of the reaction on recontact (Table 1, species 12-14). These differences correspond to the type of cell that is found in the interface (Table 2) and suggest that the archeocyte-mediated response produces a (short term) memory cell that is not part of the collencyte/gray cell-mediated response.

The correlation between the mode of allograft rejection and immune memory allows predictions to be made on those species for which secondary and third party graftings have not been done (Smith and Hildemann 1984). Barrier forming sponges (Table 1, species 1-6), would be expected to lack memory, while cytotoxic responders (Table 1, species 15-17) should exhibit accelerated, specific rejections. It should be noted, however, that *Axinella polypoides* (Table 1, species 11), which has a cytotoxic response without demonstrable memory, is a known exception to this rule.

Memory responses in sponges may be advantageous for those species that show cytotoxic reactions and have potential for repeated contact between the same pairs of individuals within the time span of short term memory (Bigger *et al.* 1982; Smith and Hildemann 1984). This would be most likely to occur in species with fast growth rates. The deposition of a barrier between allogeneic tissues would be more likely to avoid the problem of recontact and immune memory might not be advantageous in these species.

Speculations on the means by which sponges coordinate the cells involved in rejections have been advanced by several workers (Buscema and Van de Vyver 1984a,b; Smith and Hildemann 1986a,b; Zea *et al.* 1986). The mesohyl cells are directed to migrate to the area of allogeneic contact and to line up along the interface where they either secrete a barrier or initiate a cytotoxic rection. A possible controlling mechanism for this reaction might involve chemotactic factor(s) that are released by one or both sponges of a grafted pair, and result in cellular migrations with subsequent ectosomal changes. Other chemical signals released after cellular accumulations have occurred might initiate and regulate collagen secretion or the release of lytic agents. Because unmixed cellular fronts form in cytotoxically responding sponges, it has been suggested that direct cell-cell killing is not employed, and that lytic factor(s) are diffusible over short distances (Smith and Hildemann 1986b). It should be noted that these lytic factor(s) could be directed either towards any nonself cell or tissue, or alternatively, towards self in the form of a self destruct signal. The observable end result would appear the same and no investigations have been aimed at differentiating between these two possible killing mechanisms.

Variability in the magnitude and/or directionality of the responses to different allogeneic contacts has been reported in several sponges (Hildemann *et al.* 1980; Buscema and Van de Vyver 1984b; Smith and Hildemann 1984). Examples include *C. diffusa* which shows killing in both sponges (bidirectional) or only one sponge (unidirectional) of a grafted pair (Hildemann *et al.* 1980), and *A. verrucosa* which shows a range of responses from non-fusion, to apparent allogeneic fusion, to active barrier formation (Buscema and Van de Vvyer 1984b). This broad variability has been attributed to the genetic differences between the sponges (Hildemann *et al.* 1979, 1980), and may be useful in dissecting sponge graft rejections. The different types of reactions seen in *A. verrucosa* reveal two separable stages of the response: infiltration and secretion. The non-fusion response shows no anatomical changes in the areas of allogeneic exopinacoderm contact; i.e., no cellular infiltrate and no barrier formation, as if the genetic differences between parabionts were too weak to initiate the release of the chemoattractant to induce cellular infiltration. The unusual allogeneic fusion response in *A. verrucosa,* shows an infiltration of collencytes and archeocytes without barrier formation, perhaps because the signal for cellular infiltration was released, while the signal for the collencytes to secrete the collagen barrier was not. Finally, in chronic rejections, all aspects of the response are revealed; nonself recognition cues are sufficient to induce the secretion of chemical signals that are involved in collencyte infiltration and collagen secretion. A similar two-phase response may also operate in *C. diffusa* where unilateral killing occurs in some cases. Although this type of phenomenon has not been analyzed histologically, the lysis of cells in only one of two grafted sponges could result from one sponge failing to respond because of insufficient nonself stimulation to initiate secretion of the regulatory signals.

A manipulated dissection of the rejection response has only been attempted by Bigger *et al.* (1981), in *C. diffusa.* By placing a millipore filter between sensitized sponges, or by removing one sponge of a pair, it was shown that continued, direct contact of the allogeneic tissues was necessary to maintain the response. When the allogeneic tissue was replaced with self tissue, the killing response in *C. diffusa* ended and the sensitized sponge fused compatibly with the self graft. These studies indicate that direct cell contact is necessary for maintaining the reaction, perhaps by the

continued release of chemical signals, and that there are methods by which sponges can regulate or turn off a response.

ALLOGENEIC RESPONSES *IN VITRO*

The use of whole sponges to analyze allograft rejection has yielded interesting information about the immune capabilities of different species and about the cells that are involved. Although little is known about mesohyl cell function, the analysis of immune responses may initiate a more determined investigation of these cells. *In vitro* studies may be very useful in this regard.

The original reaggregation experiments were aimed at understanding how reaggregating sponge cells could separate themselves into species specific aggregates (Galtsoff 1925; Humphreys 1963; Moscona 1968). This phenomenon involves a large molecule, the aggregation factor (AF), that is found on the surfaces of all cells (Humphreys 1963). The AF acts as an intercellular glue to hold the sponge together. It is different for each species, and the ability of cells from two different species to segregate upon being mixed depends on the cross reactivity of the AFs and the AF-receptors (AF-R) on cell surfaces. But what happens in an allogeneic mixture of cells, all of which have the same AF and AF-R? It seems to depend on the capabilities of the individual species such as whether the allogeneic cells can separate themselves based on some unknown factors other than the AF, in addition to the type of allorejection response the species exhibits.

Three species have been studied in this regard, each with very different methods of allograft rejection. *Ephydatia fluviatilis* quickly and efficiently builds barriers when contacted by allogeneic tissues (Van de Vyver and De Vos 1979; Buscema and Van de Vyver 1984c). When the cells from two individuals are dissociated and mixed, chimeric cellular aggregates form, which sort out into individual-specific aggregates that settle, spread, become functional, and finally build barriers between one another. Because the input of cell numbers is not maintained, some allogeneic phagocytosis occurs in the aggregates which is the extent of the non-barrier forming allogeneic response (Van de Vyver, this volume; Van de Vyver and Buscema 1977; De Sutter and Van de Vvyer 1979). *Microciona prolifera*, which also builds barriers (Simpson 1973; Zea *et al.* 1986) forms chimeric aggregates that do not sort out into individual-specific aggregates, yet they can settle to form functional chimeric sponges with no apparent allogeneic reactions (Zea and Humphreys 1985). *Callyspongia diffusa*, which has a cytotoxic response, also forms chimeric aggregates that do not sort out when allogeneic cells are mixed, but these aggregates do not settle and are not viable beyond 48 hours. In contrast, aggregates from a single *C. diffusa* settle and become functional in 48 hours (Johnston 1988).

These *in vitro* allogeneic mixing experiments illustrate several points. First, not all species are capable of allogeneic cell sorting. When this is found in conjunction with cytotoxic responses to allogeneic contact, as in *C. diffusa*, the chimeric aggregates undergo a killing reaction. Functional organization does not seem to be necessary for the sponge cells to recognize allogeneic nonself and to react cytotoxically within the aggregate. When the ability of allogeneic cell sorting is absent from a barrier building sponge, no obvious immunological responses occur; either the ability to recognize nonself, or to secrete barriers, or both, returns only after homogeneity and functional unity are restored. Finally, it seems that the archeocytes in the barrier building

sponge *M. prolifera* either do not receive the signal(s) for, or are not capable of, allogeneic cytotoxicity even when the chimeric aggregates have been reorganized into functional sponges.

When the original xenogeneic cell sorting experiments are examined, it is clear that even though *M. prolifera* was a superior species choice for these experiments to be successful, the results led to the belief that sponges could only recognize xenogeneic cells. However, if the data from these studies are re-analyzed with allogeneic cell sorting in mind, the allogeneic interactions can be identified, even though they were not understood or believed at the time. For example, Humpreys (1963) noticed that when cells from *M. prolifera* and *Haliclona occulata* were mixed and allowed to sort out, the *H. occulata* aggregates were "short lived." If one assumes that *H. occulata* has a cytotoxic response, as does another member of the genus (C. H. Bigger, personal communications), the aggregates may have been short lived because of an allogeneic killing reaction much like that seen in *C. diffusa* aggregates.

SUMMARY AND CONCLUSIONS

The allograft rejection responses in various species of sponge have been demonstrated by many investigators, and from this accumulated information, some general statements and predictions can be made. All demosponges appear to fuse to autografts with a common set of cytological responses and anatomical changes. The exopinacoderm contact, followed by ectosomal changes and choanosome fusion, has been noted in several species. Similarly, the initial stages of allograft rejection that include nonself recognition, cellular infiltration, and accumulation, bear close resemblance in many species (Figure 3). The initial exopinacoderm contact is probably the point of nonself recognition in sponges and may also be the origin of the cellular signals that initiate the effector phase. The cell(s) responsible for this function are completely unknown. Many sponges show a migration of mesohyl cells, with or without the development of cell tracts, which accumulate to form the opposing fronts of cells at the allogeneic interface. From this point on, however, graft rejection differs in different species. The final phase of tissue separation, in most cases, is accomplished by the secretion of either collagen or lytic factor(s). Even though the signals that initiate secretion at the fronts may be similar in many sponges, the response by different types of cells at the interface appears to dictate the mechanisms by which the final rejection phase effects tissue separation.

The correlation between archeocytes, cytotoxicity and memory falls out of the accumulated data on primary, secondary and third party graft rejections on several sponges. Cytotoxic reactions occur faster and more vigorously in second set grafts, while barriers are not erected faster or larger under similar conditions. This type of response suggests that immune memory in rejected sponges with unprotected surfaces (not covered by a barrier) would be advantageous in situations of recontact from growth.

From this analysis comes the speculation of "memory immunocytes" in archeocyte-mediated reactions and suggests that adoptive transfer experiments would confer accelerated, specific rejection capabilities on naive sponges. Adoptive transfer experiments could be accomplished by fusing naive and presensitized pieces of the same sponge together. Bigger *et al.* (1981) have shown that the immune reactivity spreads throughout a sponge, which suggests that the intermixing of cells between

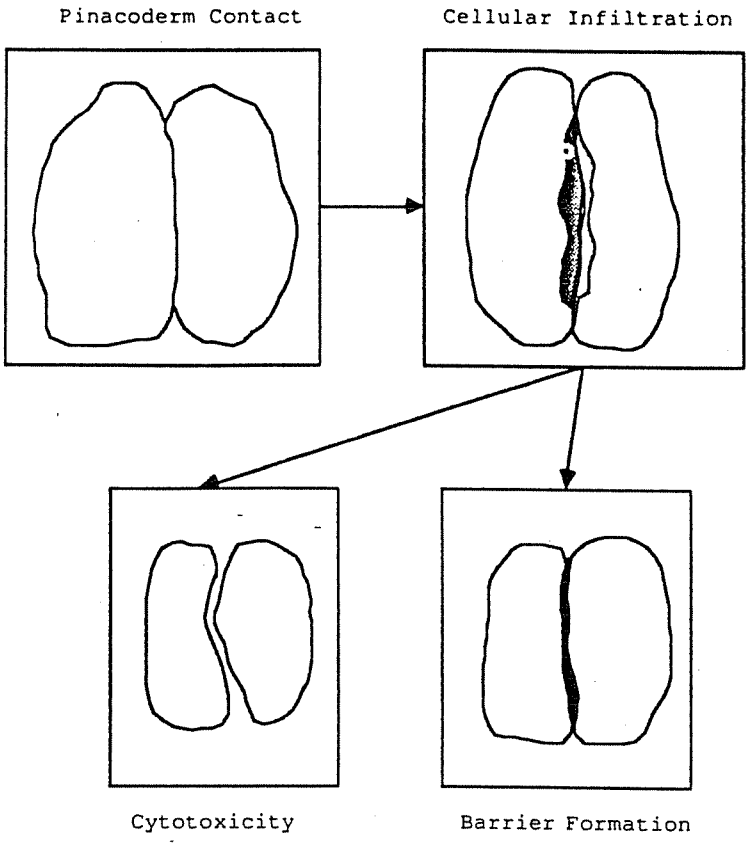

Figure 3. A Generalized Representation of Sponge Rejection Responses. Most sponges respond to exopinacoderm contact by a cellular infiltration into the graft zone. Allogeneic tissue separation is accomplished either by cytotoxic destruction of the cells near the interface or the deposition of a collagenous barrier.

autografted sponges could confer specific sensitization on the naive partner. Subsequent allografting between the adoptively transferred naive sponge and the sponge that was used for sensitization to produce the memory immunocytes, should result in specific and accelerated rejection. This grafting method of adoptive transfer has been done in *E. fluviatilis*, a fresh water, barrier building sponge (Van de Vyver 1983), but it has not been attempted on a species that shows a cytotoxic response with immune memory. Injecting dissociated cells from a presensitized sponge into a naive, histocompatible recipient has been attempted (C. H. Bigger, personal communication), however, there were some technical difficulties with this approach.

Sponges seem to be able to coordinate and regulate their rejection responses by cell surface contacts at the graft interface that induce secreted molecular signals. Continued allogeneic cell-cell contact is necessary for rejections to continue and the substitution of self tissue for allogeneic tissue results in the down regulation of the response. Chemotactic factors have been proposed for the coordination of cellular infiltration, and secreted lytic factor(s) may be acting in the cell killing phase of

cytotoxic responders. The separation of two effector stages of rejection in *A. verrucosa*, infiltration and secretion, was discernible in poor responders. It might also be possible to assess whether other species respond similarly by slowing down, stretching out and perhaps blocking their rejection reponses at various points by performing them at temperatures colder than normal for the species. This has been used commonly in analyzing the reactions of ectothermic animals.

Recent allogeneic cell mixing studies have shown that the final effector phases of allograft rejections are fundamentally different in sponges that build barriers versus those that react cytotoxically. Based on the phagocytic response in *E. fluviatilis* and the cytotoxicity in *C. diffusa*, it appears that sponge cells from some species are capable of recognizing and responding to nonself in the unorganized chimeric aggregates. Functional unity, however is required for the secretion of a collagen wall, but is not necessary for cytotoxicity. This suggests a basic organizational difference between barrier building and cytotoxically reacting sponges as far as can be discerned from the three species that have been investigated in this regard. However, the analysis of *A. polypoides* which reacts cytotoxicially and secretes collagen within the accumulated cells at the graft interface, may offer some information on the organizational requirements for the secretion of collagen versus lytic factor(s). Allogeneic mixing studies of this genus would aid greatly in analyzing the reactions of various sponge cells *in vitro*.

In vitro studies have also shown that allogeneic mixtures of cells from some species are capable of sorting out while others are not. Since the AF and AF-R found in all individuals of one species are the same, allogeneic sorting must be due to a different and unknown set of molecules. Investigations into this phenomenon have not been undertaken.

The study of sponge histocompatibility reactions has progressed immensely in recent years. It has gone from the belief that sponges could only recognize xenogeneic cells to speculations on regulatory mechanisms, and on the cell surface molecules and secreted factor(s) that are involved in an effective defense system that can recognize and discriminate between a large variety of nonself contacts and challenges. These sorts of capabilities in sponges are of great importance in the natural environment of the animal. Not only is it necessary to defend and perhaps expand one's space for the purposes of growth, but it is also important to protect self from microbial infection. Sponges probably come into contact with nonself most commonly through filter feeding, and thus must be able to discriminate between food, nonfood and pathogens. Clearly the sponges have evolved to be very efficient in accomplishing these tasks.

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