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Clone-specific cellular recognition in a sea anemone

(nematocytes/allogeneic recognition/cell surface markers)

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ABSTRACT **A highly specific cellular recognition system, capable of distinguishing between syngeneic and allogeneic tissue, exists in *Anthopleura elegantissima*, a sea anemone that lives in clonal colonies and attacks foreign clones. During the attack, specialized surface protrusions (acrorhagi) are used for stinging. The recognition process was studied by presenting various tissues to the surface of inflated acrorhagi and observing whether nematocyst discharge occurred. Nematocyte excitation required direct contact of the acrorhagus with foreign tissue and is presumably mediated by cell surface receptors. Most foreign anthozoans were excitatory, but intact syngeneic individuals, organisms other than anthozoans, and inanimate objects consistently failed to elicit discharge. When the intact surface of an excised tentacle from one anemone was presented to the acrorhagus of another, discharge occurred in 101 of 102 allogeneic combinations; more than 50 tests with tentacles from clone mates (i.e., syngeneic combinations) were all negative. No evidence for specific immunological memory was found. It is suggested that clonal recognition depends upon genetically determined chemical markers in the surface membrane of the epithelial cells; these are assumed to differ between clones although, in rare cases, allogeneic clones may have similar markers.**

The sea anemone *Anthopleura elegantissima* lives in clonal colonies. Francis (1, 2) found that individuals would exhibit a complex pattern of aggressive behavior when put in contact either with members of other clones or with other species of anthozoan; syngeneic individuals were not attacked. An important feature of the aggressive behavior is the inflation and protrusion of specialized organs known as acrorhagi; these are richly provided with large nematocysts and are used to sting the opponent. The stinging response could be evoked experimentally by touching an inflated acrorhagus with excised tissue from a foreign anemone (2).

The present paper describes the acrorhagial stinging response in detail. This response is of particular interest because it appears, for reasons given below, to represent a highly specific case of cellular recognition occurring in the ectodermal cell layer. The specificity of the acrorhagial stinging response was examined in a number of experiments and compared to that of the oral tentacles. The ability of acrorhagi to distinguish between tissues from a large number of clones was tested by using Francis' (2) technique of presenting excised tentacles. Experiments were also undertaken to test for memory in the immunological sense and to see whether acrorhagial recognition of syngeneic individuals might be dependent upon habituation (cf. ref. 3).

MATERIALS AND METHODS

Clones of *A. elegantissima* were identified as described (1). They were collected in the shore preserve of the Scripps Institute of Oceanography (La Jolla, CA) and maintained in shallow aquaria supplied with running sea water at 17°C; they were fed pieces of mussel.

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Experimental anemones were placed in small dishes of sea water under a binocular microscope and induced to expand their acrorhagi by contact with an allogeneic individual. In order to determine whether acrorhagial nematocytes were excited by a particular substrate, test objects were touched for five 1-sec periods against the tip of each of three fully inflated acrorhagi (partially inflated acrorhagi were not used). When it occurred, nematocyst discharge was massive and simultaneous and could be readily observed. The response of nematocytes in the oral tentacles was assayed by touching inflated tentacles in a similar manner and observing whether the tentacle adhered to the test object; numerous discharged nematocysts could be observed microscopically on test objects to which a tentacle had adhered.

Anemones used in the experiment on specific immunological memory were collected from the border (1) between two adjacent clones on an isolated piling; no other clones were present on the piling. Individuals on the border had numerous large acrorhagi that were presumed to be a consequence of repeated combat with adjacent members of the other clone (4); anemones distant from the interclonal border had only a few poorly developed acrorhagi.

RESULTS

In an initial experiment, the response of nematocytes on the acrorhagi was compared to that of nematocytes present on the oral tentacles. Neither group responded to intact syngeneic individuals (Table 1). Tentacular nematocytes discharged against a wide variety of animal substrates ranging from sponges and other anemones to fishes, but the acrorhagial nematocytes responded only to allogeneic individuals and certain other species of anthozoan (cf. ref. 2). In addition, clean inanimate objects such as metal forceps, glass rods, or polythene rods consistently failed to excite the nematocytes on the acrorhagi, although occasionally they did excite those on the tentacles. It thus seemed clear that the recognition mechanism associated with acrorhagial nematocyte discharge was significantly more specialized than that associated with the discharge of tentacular nematocytes.

The discharge of acrorhagial nematocytes was massive and simultaneous, involving usually from one-fifth to four-fifths of the nematocyte-bearing tissue at the end of the acrorhagus; frequently, nematocytes more than 1–2 mm from the point of contact with the foreign stimulus would be involved in the explosion. Discharge could only be induced on physical contact of the tip of the acrorhagus with the appropriate substrate, as found by Francis (2). Allogeneic tissue would not elicit discharge when held for 5–10 min at 0.5–2 mm from the tip of the acrorhagus; on the other hand, discharge usually occurred within 2–3 sec of contact. It was notable that glass rods coated with external mucus from allogeneic individuals failed to excite the acrorhagial nematocytes; in contrast, the outer surfaces of excised pieces of allogeneic tentacle or column were excitatory. This suggested that discharge required contact with the surface of the foreign anemone's epithelial cells.

Table 1. Response of acrorhagial nematocytes and tentacular nematocytes to contact with external surface of various organisms

Stimulus	Response	
	Tentacular	Acrorhagial
Porifera:		
Leucoselenia nautila	+	−
Tethya aurantia	+	−
Cnidaria (Hydrozoa):		
Obelia sp.	+	−
Tubularia crocea	+	−
Cnidaria (Anthozoa):		
Anthopleura elegantissima (clone mate)	−	−
A. elegantissima (not a clone mate)	+	+
A. artemisia	+	+
A. xanthogrammica	+	+
Anthopleura sp.	+	+
Aiptasia californica	+	+
Epiactis prolifera	+	+
Metridium senile	+	+
Corynactis californica	+	+
Astrangia lajollaensis	+	+
Eugorgia rubens	+	−
Ectoprocta:		
Membranipora membranacea	+	−
Annelida:		
Marphysa sanguinea	+	−
Arthropoda:		
Elasmopus sp.	+	−
Pollicipes polymerus	+	−
Lophopanopeus frontalis	+	−
Echinodermata:		
Amphipholis pugeta	+	−
Strongylocentrus purpuratus	+	−
Mollusca:		
Littorina planaxis	+	−
Mytilus edulis	+	−
Hemichordata:		
Styella ?plicata	+	−
Vertebrata:		
Paralabrax clathratus	+	−

+, Nematocyst discharge was observed; −, no discharge was observed.

The external surface of syngeneic individuals or excised syngeneic tentacles (>50) consistently failed to elicit discharge. Discharge against syngeneic tissue could only be induced after mechanical damage. In 5 of 20 cases the freshly cut end of a syngeneic tentacle caused discharge. This perhaps was due to enzymic alteration of syngeneic markers, to the exposure of active substances normally hidden, or to enzymic activity directly against the tip of the acrorhagus.

It seemed that the failure of intact syngeneic individuals to elicit discharge could be ascribed either to some genetically determined surface characteristic (cf. ref. 1) or to some form of habituation resulting from prolonged contact between anemones. In order to distinguish between these two possibilities, 13 syngeneic individuals were removed from their clone mates and maintained alone in separate aquaria for 23 days. At the end of this period the intact surfaces of tentacles excised from isolated anemones were touched against the inflated acrorhagi of a syngeneic anemone that had remained in contact with clone mates. On no occasion did acrorhagial discharge occur. This indicated that the failure to sting syngeneic individuals was not dependent upon continuous contact between clone mates. It was consistent with the hypothesis that clonal recognition is genetically based and suggested that *A. elegantissima* was able to distinguish between conspecifics by means of clone-specific surface markers.

Francis (2) found that 75 different allogeneic combinations all induced aggressive behavior, but it is not clear from her results whether she also tested for acrorhagial discharge. The diversity of surface markers used in acrorhagial recognition was therefore examined by using 102 allogeneic combinations. The experiment involved testing the acrorhagial stinging response of 3 different, incompatible clones to members of 34 other clones that had been collected about 400 m from the test clones. In 101 of 102 cases, the foreign clone induced nematocyst discharge; in only 1 case did acrorhagial contact with a foreign clone fail to induce discharge. The two clones involved in the latter case were very differently colored (one clone had a brownish column and yellowish tentacles with pink tips, whereas the other had a greenish olive column and brown tentacles with pale tips) and thus clearly allogeneic (1). Subsequent experiments showed that the acrorhagial nematocytes of both clones consistently failed to discharge against each other, even though they were quite capable of stinging other foreign clones; furthermore, individuals of the two clones did not show an aggressive response toward each other even when left in contact for more than 24 hr. These results suggest that each clone possesses different surface markers that are used in interclonal recognition; on rare occasions, genetically different clones may possess similar clonal markers and be unable to recognize each other as allogeneic.

Parenthetically, it is worth noting that the oral tentacles of the two compatible allogeneic clones mentioned above frequently adhered to each other; such an occurrence was never observed during contact between syngeneic individuals, and it indicated that nematocyst discharge was taking place. Bearing in mind that the recognition system associated with the discharge of tentacular nematocytes is quite different from that associated with acrorhagial nematocytes (Table 1), such a phenomenon is perhaps not unexpected; presumably, the tentacular nematocytes were responding to differences in surface substances not involved in interclonal recognition.

An interesting possibility was that specific immunological memory might be involved in interclonal recognition. Five syngeneic individuals were selected whose previous aggressive experience appeared to have been confined to members of one particular foreign clone as judged by their position in the natural habitat. They were presented with excised allogeneic tentacles and their response was quantified by counting the number of times each tentacle had to be applied (duration of application, 1 sec) against a fully inflated acrorhagus before it elicited discharge. Fifteen tentacles from the clone with which the test anemones had had previous aggressive experience each required one to seven applications (mean, 2.00) before they induced discharge. Fifteen tentacles from each of two other incompatible clones not previously encountered required one to three (mean, 1.27) and one to five (mean, 1.80) applications, respectively, before nematocyst discharge occurred. Thus, there was no evidence that repeated aggression against one particular clone specifically enhanced the aggressive response to that clone.

DISCUSSION

No detailed explanation of the physiological mechanism underlying the discharge of acrorhagial nematocytes in *A. elegantissima* has been presented. The results presented above are in agreement with Francis' (2) observation that physical contact is required between the acrorhagus and an appropriate substrate before nematocyst discharge will occur. Discharge is not an invariable consequence of aggressive behavior and it

only occurs when the acrorhagial tip touches an object of suitable chemical composition; furthermore, discharge is relatively local and usually occurs rapidly after contact. These observations suggest that the excitation of acrorhagial nematocytes is governed by a cellular recognition system associated with surface ectodermal cells (cf. ref. 5); the chemical receptors involved are probably situated on the ciliary cones that cover the end of the acrorhagus (6).

The specificity of the acrorhagial response in *A. elegantissima* was first examined by Francis (2), who found that clone mates, hydrozoans, molluscs, and echinoderms did not elicit discharge whereas all foreign clones and other species of anthozoan were excitatory. The results of the present study confirmed that the intact external surface of syngeneic individuals was not stung. On the other hand, damaged syngeneic tissue was sometimes found to elicit discharge; this may explain Bigger's (3) observation that syngeneic tissue is occasionally stung in the closely related *A. krebsi*. The majority of allogeneic clones induced stinging in *A. elegantissima;* in one case, however, two allogeneic clones were compatible and did not excite each other's acrorhagial nematocytes. As in *A. krebsi* (3), most but not all foreign species of anthozoan were capable of inducing the discharge of acrorhagial nematocysts. Discharge could not be induced, however, by a wide variety of organisms other than anthozoans or by certain inanimate objects. The acrorhagial recognition system is clearly different from the less-specialized system associated with nematocytes on the oral tentacles. Tentacular nematocytes did not respond to syngeneic individuals but discharged against numerous other creatures ranging from sponges and anemones to fishes.

The receptors on the acrorhagi thus appear only to be excited by allogeneic or xenogeneic anthozoans and not by foreign material in general. It is possible that this restriction in recognition may be analogous to the major histocompatibility restriction of vertebrates (7). In the latter, a foreign antigen does not induce a response unless associated with histocompatibility markers signifying self. Perhaps in both cases the response is restricted to a molecular configuration indicating "altered self" rather than "totally foreign."

Recognition systems associated with the discharge of nematocysts used in aggression have not been examined in most cnidarians. However, the results of Bonnin (8), Francis (2), Bigger (3), and Purcell (9) suggest that systems of specificity similar to that associated with the acrorhagi of *A. elegantissima* may occur in *Actinia equina, Anthopleura artemisia, Anthopleura krebsi,* and *Metridium senile* but not in *Anthopleura xanthogrammica*. The organs involved in aggression in the coral *Montastrea cavernosa* (10) seem significantly less specific in their response than do those of *A. elegantissima*.

The distinctive behavior pattern involved in aggression in *A. elegantissima* is usually initiated on contact of the oral tentacles with a foreign antozoan (1, 2). This suggests that there is a population of receptors on the oral tentacles that is of specificity similar to that on the acrorhagi; the former are presumably independent from the receptors usually involved in the excitation of tentacular nematocytes because numerous organisms can elicit nematocyst discharge without inducing aggressive behavior. However, one cannot assume at present that substances eliciting aggressive behavior are necessarily the same as those that excite acrorhagial nematocytes. Bigger (3) found that certain substrates would induce aggression in *A. krebsi* but would not cause acrorhagial discharge.

Francis (1, 2) suggested that clonal recognition was genetic but did not provide clear experimental evidence against the hypothesis that some form of habituation might exist between clone mates. The present experiments showed that isolated syngeneic individuals remained incapable of eliciting acrorhagial discharge by their clone mates. This result supports Francis' hypothesis and is analogous to that obtained by Bigger (3) in *A. krebsi:* Bigger examined the effect of habituation on aggressive behavior rather than on the acrorhagial stinging response and found that habituation was not required to prevent clone mates from attacking each other.

In the above experiments, no evidence could be found of specific immunological memory being involved in the recognition of allogeneic clones. Francis (4) demonstrated that repeated aggression leads to the development of larger and more numerous acrorhagi. This may be regarded as a form of nonspecific memory in the sense that repeated contact with a particular clone enhances the development of the structures involved in aggression; however, the acrorhagial response to the previously encountered clone does not seem to be faster or greater than that to other allogeneic clones.

It is noteworthy that the external mucus of incompatible allogeneic anemones was incapable of exciting acrorhagial nematocytes. This suggests that the receptors associated with acorhagial discharge respond to surface substances bound to the plasma membrane of the foreign anemone's ectodermal cells. Acrorhagial discharge does not appear to be a consequence of the foreign anemone stinging the acrorhagus as previously suggested (8): washed allogeneic tissue previously fixed in formalin retains its excitatory capabilities (6). Allogeneic mucus was also found to be incapable of eliciting aggressive behavior in *A. elegantissima* as in *A. krebsi* (3); unfortunately, Bigger did not test whether mucus would elicit the discharge of acrorhagial nematocysts in the latter species.

In summary, the cellular recognition system associated with the discharge of acrorhagial nematocytes in *A. elegantissima* is remarkably specific and enables the anemone to distinguish between syngeneic and allogeneic individuals. Acrorhagial nematocytes are excited by most foreign anthozoans, but they are not excited by intact syngeneic individuals, organisms other than anthozoans, or inanimate objects. Clonal recognition appears to be based upon genetically determined chemical surface markers bound to the plasma membrane of the anemone's ectodermal cells. Contact between syngeneic individuals is not excitatory because they possess similar surface markers; on the other hand, allogeneic individuals generally have different clonal markers and thus excite the receptors associated with the discharge of acrorhagial nematocytes. On rare occasions, genetically different clones may possess similar clonal markers and so not recognize each other as allogeneic.

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