Specific Tissue Graft Rejection in Earthworms

Abstract. *Earthworms are capable of destroying antigenic tissues. Autogeneic transplants healed in regularly and remained permanently viable. Xenografts, by contrast, were cicatrized but eventually rejected. Intrafamilial transplants survived longer than interfamilial ones. Xenografts and autografts placed in the same graft bed were joined to each other but xenografts were later destroyed although autografts were not. Two xenografts from* Eisenia *to* Lumbricus, *performed simultaneously, showed survival endpoints similar to a single xenograft. A 5-day interval between first- and second-set grafting led to an accelerated rejection of both transplants. First-set* Allolobophora *transplants to* Lumbricus *performed simultaneously with second-set* Eisenia *grafts were destroyed at a time different from either of the two* Eisenia *transplants. A single* Allolobophora *transplant to* Lumbricus *was rejected at survival times equivalent to* Allolobophora *along with two* Eisenia *transplants.*

The evolutionary origin of the ability to reject tissue transplants has been controversial. With the demonstration of xeno- and allograft destruction in an invertebrate, the earthworm (*1*, *2*), it is clear that transplant rejection indeed evolved earlier in the evolutionary scheme than with the appearance of the first vertebrates. Lampreys and hagfishes are both jawless fishes; one view has held that lampreys were the most primitive vertebrates endowed with the capacity to recognize and destroy tissue allografts (*3*). However, hagfishes regularly reject allografts in a chronic fashion (*4*). The ability to destroy tissue grafts in earthworms is highly specific, that is, host worms can distinguish sharply between antigenic tissue of various other genera of oligochaete annelids.

Four genera of annelid worms (families Lumbricidae, Eudrilidae), *Lumbricus terrestris, Eisenia foetida, Allolobophora trapezoides,* and *Eudrilus eugeniae* were used. All features of worm maintenance, grafting, the postoperative period, and the criterion of graft rejection have been described (*1*).

Autografts performed alone even with the reversal of the anterior-posterior polarity were never destroyed (*1*). Autografts and xenografts in the same graft bed healed together, but xenografts were eventually rejected leaving a permanently surviving autograft.

Intrafamilial transplants were recognized although graft destruction was delayed as compared with interfamilial transplants (Table 1). Intrafamilial grafts between three genera of Lumbricidae showed consistently longer survival times compared with interfamilial transplants involving a genus of the family Eudrilidae. These observations corroborate the gross criteria for the establishment of certain taxonomic designations.

When two *Eisenia* grafts were transplanted from the same donor to a host *Lumbricus*, survival time was affected by the time of challenge of the two grafts. A single graft was rejected at a mean survival time of 26 days (Table 2). Two transplants grafted at the same time to *Lumbricus* were destroyed simultaneously. However, when two transplants from the same donor were grafted to *Lumbricus* 5 days apart, both first and second transplants were destroyed in an accelerated fashion; a significant difference was found between these two survival times and between each of those times and that of a single graft ($P < .05$).

An *Eisenia* graft followed by 5 days with a second-set transplant shortened the survival time of both transplants when compared with single grafts ($P < .05$); a significant difference between the survival times of both transplants was found. An additional third-party graft from *Allolobophora* to *Lumbricus* resulted in its destruction at a survival time (43 days) different from the *Eisenia* transplants but equivalent to that of grafts of *Allolobophora* to *Lumbricus* alone (38 days) (Table 1). This consistent survival time demonstrated the independence of immunologic responses to *Allolobophora* and *Eisenia* antigens and hence the specificity of the rejection response of *Lumbricus*.

Xenogeneic transplants exchanged between members of the same earthworm family had longer survival times than grafts between members of different families (Table 3). One would predict that antigenic diversity would be greatest between families but less within families where presumably a greater number of antigens are shared. This is supported by the survival times of familial transplants, a situation analogous to that in poikilothermic vertebrates such as salamanders where inter-

Table 1. Survival times of first-set xenografts exchanged between several earthworm genera.

Type of transplant	Grafts (No.)	Mean survival time (days)	Range (days)
Intrafamilial (Lumbricidae)			
Lumbricus → *Eisenia**			
(autograft control)		---	---
(xenograft)	56	33.60†	12–69
		33.60‡	12–90
Eisenia → *Lumbricus*	87	26.05	8–81
Allolobophora → *Lumbricus*	61	38.00	10–78
Allolobophora → *Eisenia*	39	35.28	16–71
Lumbricus → *Eisenia*	25	34.50	14–105
Interfamilial (Eudrilidae → Lumbricidae)			
Eudrilus → *Lumbricus*	49	13.34	7–23
Eudrilus → *Eisenia*	15	17.40	11–32

* The *Eisenia* autograft and the *Lumbricus* xenograft were placed in the same graft bed. Autografts survived permanently. † Xenograft anterior; autograft posterior. ‡ Autograft anterior; xenograft posterior.

Table 2. Survival times of first- and second-set intrafamilial (Lumbricidae) xenografts exchanged simultaneously and with a 5-day interval between several earthworm genera. Zero indicates no interval between first and second transplant; +5 indicates 5 days between first and any subsequent grafts.

Type of transplant	Grafts (No.)	Set	Mean survival time (days)	Range (days)
0				
Eisenia → *Lumbricus* ← *Eisenia*	38		32.77	10–105
+5				
Eisenia → *Lumbricus* ← *Eisenia*	64	First	17.8	7–60
		Second	15.7	4–32
+5				
Eisenia → *Lumbricus* ← *Eisenia*	92	First	18.1	8–72
		Second	15.3	4–50
+5				
↖ *Allolobophora*	92	First	43.4	5–81

familial xenografts were exchanged (*5*).

Graft rejection may be mediated by coelomocytes which seem to congregate around and in xenografts reaching maximum numbers approximately 5 days after grafting (*6*). Moreover, that some of these cells were observed during healing suggests that cell involvement in initial general nonspecific reactions (for example, healing of wounds) was associated with all transplants. Thus, with xenograft rejection and autograft acceptance, one may infer specificity in coelomocyte recognition of the difference between "self versus not self" antigens. To test the capacity of worms to distinguish between these antigens, an autograft and xenograft were transplanted simultaneously to the same graft bed of a *Lumbricus* host. Cells involved in nonspecific reactions during the early phases of healing were thus confronted simultaneously with auto- and xenogeneic antigens. Both grafts healed together; the xenograft was destroyed later, but the autograft remained permanently viable.

Table 3. Statistical analyses of graft survival times (data from Tables 1 and 2). The .05 significance level was accepted as indicating significant population difference. The (W) indicates use of Wilcoxon matched pairs signed rank test, all others analyzed with the Mann-Whitney *U* test (two-tailed) (*8*); +5 indicates an interval of 5 days between the first- and second-set grafting.

Direction of grafting compared	*P*
**Lumbricus* → *Eisenia* **Lumbricus* → *Eisenia* xenograft ↑ ↑ autograft	.32
Allolobophora → *Lumbricus* *Eisenia* → *Lumbricus*	<.00003
Eudrilus → *Lumbricus* *Eisenia* → *Lumbricus*	<.00003
Allolobophora → *Lumbricus* *Eisenia* → *Lumbricus*	.0001
Eudrilus → *Eisenia* *Lumbricus* → *Eisenia*	.002
Eudrilus → *Eisenia* *Allolobophora* → *Eisenia*	.00014
Allolobophora → *Eisenia* *Lumbricus* → *Eisenia*	.40
Eisenia* → *Lumbricus* **Eisenia* → *Lumbricus* ← *Eisenia	.38
Allolobophora* → *Lumbricus* +5 *Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolophora	.19
+5 **Eisenia* → *Lumbricus* ← *Eisenia*	.96
+5 **Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolobophora*	
+5 *Eisenia* → *Lumbricus* ← *Eisenia**	.96
+5 *Eisenia* → *Lumbricus* ← *Eisenia** ↑ +5 *Allolobophora*	
**Eisenia* → *Lumbricus* +5 **Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolobophora*	.00014
Eisenia* → *Lumbricus* +5 *Eisenia* → *Lumbricus* ← *Eisenia ↑ +5 *Allolobophora*	<.00003
Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolobophora +5	<.00003 (W)
Eisenia* → *Lumbricus* ← *Eisenia* *Eisenia* → *Lumbricus* ← *Eisenia +5	<.00003
**Eisenia* → *Lumbricus* ← *Eisenia* ↑ +5 *Allolobophora* +5	.0013 (W)
Eisenia → *Lumbricus* ← *Eisenia** ↑ +5 *Allolobophora*	

* Indicates which samples were compared.

The experiment which indicated the high degree of specificity of earthworm immune competence involved transplants from *Eisenia* and *Allolobophora* to *Lumbricus* hosts. Two *Eisenia* transplants showed a shortened rejection time, while a single *Allolobophora* transplant was destroyed independently; a control *Allolobophora* graft on a *Lumbricus* host was rejected at the same time. A host *Lumbricus* with two xenotransplants grafted simultaneously but in different locations showed a survival time equivalent to a single *Eisenia* graft to *Lumbricus*.

The earthworm's ability to reject transplants, in itself, had no apparent survival value in evolution. Graft destruction has only provided a convenient model for demonstrating biological specificity. Yet, in the context of the phylogeny of immunity we have revealed, for the first time, a primitive immune system in an invertebrate that may be cell-mediated and prototypic. The apparent inability of earthworms to synthesize substances in response to various bacterial antigens suggests that humoral immunity evolved in other invertebrate phyla (*7*) and primitive vertebrates (*4*). Further clarification of anamnestic responses to tissue transplants would confirm our views that at least two of the parameters of adaptive immunity, namely specificity and memory, did not evolve exclusively with the lower vertebrates.

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References and Notes

1. E. L. Cooper, *Transplantation* **6**, 322 (1968).
2. ———, *ibid.* **8**, 220 (1969); *J. Exp. Zool.* **171**, 69 (1969); *J. Nat. Cancer Inst.* **31**, 655 (1969).
3. R. A. Good and B. W. Papermaster, *Advan. Immunol.* **4**, 1 (1964).
4. W. H. Hildemann and G. H. Thoenes, *Transplantation* **7**, 506 (1969).
5. N. Cohen, *ibid.*, p. 332.
6. E. L. Cooper, in preparation.
7. R. Acton and E. E. Evans, *J. Bacteriol.* **95**, 1260 (1968); E. L. Cooper, R. T. Acton, P. Weinheimer, E. E. Evans, *J. Invert. Pathol.*, in press.
8. S. Siegel, *Non-parametric Statistics* (McGraw-Hill, New York, 1956).
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Pathotoxin-Induced Disease Resistance in Plants

Abstract. *Primary leaves of bean plants treated with nonphytotoxic concentrations of the pathotoxin victorin were rendered highly resistant to two plant viruses. Leaves treated with higher concentrations of victorin became necrotic. These effects on plants that are resistant to victorin and to the fungus that produces it lend support to the hypothesis that activation of a defensive self-repair mechanism may account for resistance to this highly selective pathotoxin.*

Pathotoxins are substances of biological origin which play causal roles in plant diseases (*1*). A well-known example is victorin, a pathotoxic product of *Helminthosporium victoriae* Meehan and Murphy, the fungus which causes Victoria blight of oats (*2*). Very small quantities of victorin applied to susceptible oats cause pathological changes in permeability, transpiration, and respiration which are followed by visible disease symptoms and death of the plants (*2*, *3*). Much larger quantities cause similar pathological changes in the physiology of resistant oat tissues, but, in these, lethal effects do not follow (*4*). This ability of resistant tissues to respond and recover led to the hypothesis that activation of a defensive self-repair mechanism may account for resistance to victorin (*5*). Once activated, such a defense mechanism might render plants resistant to other pathogenic agents. To test this possibility we studied the effect of victorin on the reaction of the bean *Phaseolus vulgaris* L. cv Pinto to two viruses which induce necrotic lesions on primary leaves.

The source of victorin was a single lot of culture filtrate which, undiluted, contained 10,000 unit/ml in the standard root-growth test (*6*). When used in the crude form, the filtrate was diluted