heteroduplex may be stable for at least 800 bp. In any case, a calculation similar to the one described above indicates that the 3′ portions of X blocks have diverged for as long as 40 million years since the last gene correction event. This value is close to that of the Y blocks. We proposed earlier that a large DNA insertion/deletion (200 to 1000 bp) may inhibit gene correction (*4*). Hence, the genetic rearrangement events that generated the nonhomology blocks I and II in the human adult α-globin gene region may have occurred 40 million years ago.

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23 April 1984; accepted 27 July 1984

Rapid Expulsion of *Trichinella spiralis* in Suckling Rats

Abstract. *Orally administered* Trichinella spiralis *muscle larvae were rapidly expelled by rat pups suckling an immune dam. The immunity was delivered in the milk; substantial resistance was conferred on normal rat pups suckled for only 24 hours by a* Trichinella*-immune foster mother. The pups were protected by oral or systemic administration of specific serum antibodies. When infused into a normal lactating dam, these antibodies accumulated in the serum of her suckling pups.*

The immune response to the enteric helminth *Trichinella spiralis* involves several distinct but synergistic responses to different phases of the parasite's life cycle (*1*). A dramatic response in appropriately immunized adult rats is manifested in the prompt rejection of up to 99 percent of orally administered *T. spiralis* muscle-stage larvae (*1*). This rapid expulsion is a major defensive response against reinfection; yet the mediator of the expulsion process has not been identified, nor is it known how infectious larvae are displaced from their intestinal niche. To express rapid expulsion, adult rats must be exposed to the enteric phase

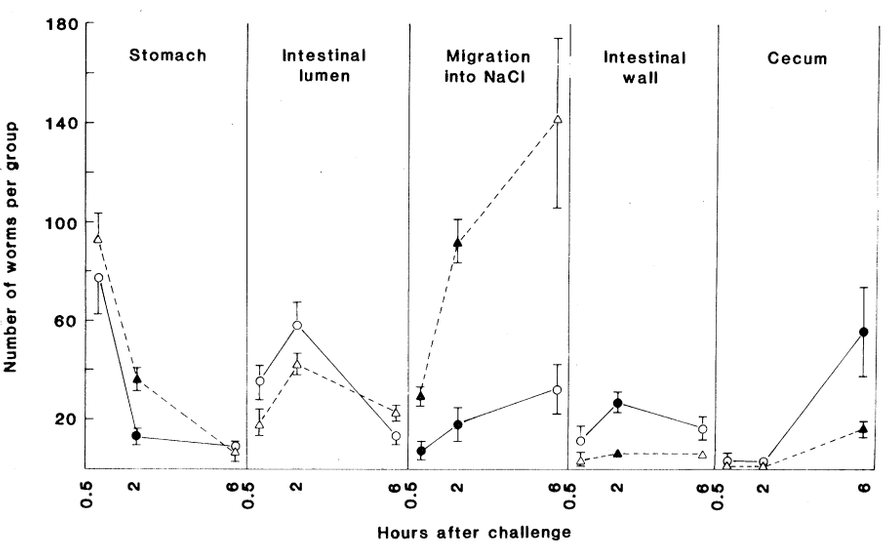

Fig. 1. Kinetics of rapid expulsion in infant rats. Five compartments of the gastrointestinal tract of 14- to 16-day-old AO rats were examined for *T. spiralis* 30 minutes, 2 hours, or 6 hours after oral challenge with 200 *T. spiralis* larvae. Dams were infected with 1000 larvae 28 days before being bred. Pups were separated from the dams 2 to 4 hours before being challenged. They were killed by cervical dislocation and the stomach, intestine, and cecum were removed. The intestines were rinsed with 0.85 percent NaCl and the wash was saved for enumeration of luminal worms. They were then slit open and incubated in 0.85 percent NaCl for 5 hours at 37°C. Worms that migrated into the medium were collected and counted. The intestinal tissue was digested overnight at 37°C with 1 percent pepsin HCl; worms freed by this procedure were also counted. The stomach and cecum were rinsed with 0.85 percent NaCl and the wash was examined for worms. Worms appearing in the cecum had been expelled, while worms appearing in the migratory compartment were considered to have established themselves in the intestine. The mean number of worms per compartment for two or three pups from each of three to six litters was calculated for each time point. Significant differences between treatment groups were determined with the Wilcoxon rank-sum test. For simplicity, pooled means and standard errors are shown for each point. Symbols: (△) nonimmune pups and (○) immune pups; shading indicates significant differences ($P < 0.05$) between groups.

of the parasite's life cycle. The experiments described here show that in the newborn rat, previous intestinal exposure to a parasite is not required for the rapid expulsion of *T. spiralis* and that antibodies can mediate such expulsion.

Culbertson (*2*) reported that immunity to *T. spiralis* in suckling neonates was manifested by reduced intestinal worm burdens 4 days after challenge. Similar results were obtained in mice by Duckett *et al.* (*3*). In our experiments AO uniparous or virgin female rats were infected orally with 1000 *T. spiralis* muscle-stage larvae and bred 4 weeks later. Suckling pups were challenged with 200 *T. spiralis* larvae at 14 to 16 days of age. We found that 70 to 99 percent of the larvae were expelled from the intestines of these pups within 24 hours and that the intestinal worm burden did not decline significantly thereafter. We determined the kinetics of worm rejection by killing pups born to infected and uninfected dams at 30 minutes, 2 hours, or 6 hours after challenge and counting the larvae in the stomach, lumen of the small intestine, and cecum. Measurements were also made of the number of larvae capable of migrating from the small intestine into saline over 5 hours and the remainder released from the intestinal wall by digestion with pepsin.

Results obtained with five control and six immune litters are shown in Fig. 1. Although similar numbers of worms were recovered from immune and control litters at 0.5 and 2 hours, the location of the worms in the intestines differed in the two groups. For instance, as early as 30 minutes after challenge there were fewer migratory larvae in the intestines of rats suckled by immune mothers. Also, many of the worms that penetrated the intestinal epithelium of immune rats failed to migrate into saline. In control rats most penetrating larvae retained their capacity to migrate. This finding suggests that, in immune rats, the worms were inhibited from migrating or that the "immune intestine" was modified in a way that impeded the parasite's mobility. The appearance of a significantly larger number of worms in the ceca of immune pups than in the ceca of normal pups at 6 hours confirmed that worms were expelled from the intestines of immune pups and that these animals were protected.

In eight litters worms in the intestinal lumen were examined for association with mucus. To render mucus-associated worms visible we compressed the mucus between glass cover slips and slides. Although the number of worms in the lumen did not differ significantly between immune and control litters, 99 percent (range, 96 to 100 percent) of worms in the intestinal lumina of immune groups examined (n = 4) were tightly bound by mucus, while in nonimmune groups (n = 4) only 38 percent (range, 10 to 59 percent) were mucus-associated. Thus two phenomena were

Table 1. Effect of passive transfer of serum Ig on worm burdens in suckling rats. Pups received 5 mg of serum Ig (see legend to Fig. 3) twice daily for 3 days before being challenged. Intestinal migratory worms were counted 18 hours after the challenge (values are means ± standard errors).

Treatment	Route of administration	Number of rats per group	Intestinal worm burden per litter
Serum Ig from normal animals	Intraperitoneal	6	126 ± 9
	Oral	4	123 ± 13
Serum Ig from immune animals	Intraperitoneal	5	47 ± 13*
	Oral	5	54 ± 12*
Fostering by infected dam†		9	48 ± 6

*Significantly different from values for recipients of serum Ig from normal rats ($P < 0.05$, Wilcoxon rank-sum test). †The dam received 1000 muscle larvae 28 days before being bred.

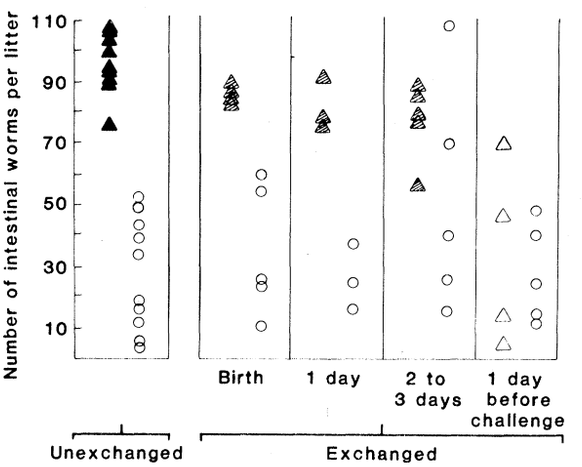

Fig. 2. Transfer of maternal immunity against *T. spiralis* to suckling rats. Rat dams and suckling rats were infected and challenged as described in the legend to Fig. 1; however, the pups were returned to the dams soon after challenge and all were killed after 18 hours. Litters of equivalent size (n = 3 to 10; mean, 5.7) were exchanged between infected and uninfected dams at the tin es shown after birth or 24 hours before challenge with *T. spiralis*. The number of migratory worms was used as the measure of immunity in this and subsequent experiments. Symbols: (○) litters suckling on immune dams when challenged and (△) litters suckling on normal dams when challenged. Cross-hatched triangles indicate treatment groups that did not differ significantly from the unexchanged, nonimmune litters (▲). Exchanged treatment groups (△, ○) harbored significantly fewer worms than the unexchanged, nonimmune group ($P < 0.01$, Studentized range test).

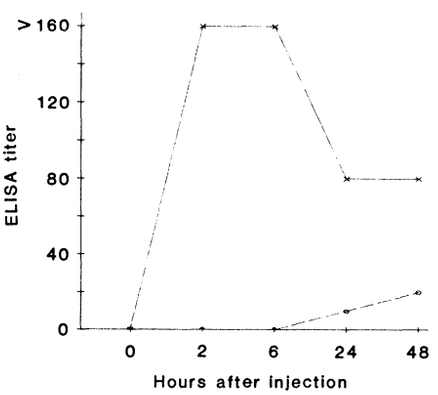

Fig. 3. Serial transfer of *T. spiralis* antibodies to suckling rats. Serum was collected from normal rats or from males and females (3 weeks postpartum) that had been infected with *T. spiralis* 9 to 11 weeks earlier. Serum was heated to 56°C for 30 minutes and immunoglobulin was precipitated three times with 33 percent saturated $(NH_4)_2SO_4$ and dialyzed against 0.85 percent NaCl. Immunoglobulin from normal serum or antiserum to *T. spiralis* (predominantly IgG by immunoelectrophoresis) (25 mg) was injected intravenously into each lactating dam approximately 14 days after parturition. Suckling pups were bled from the retro-orbital sinus and dams from the tail immediately before injection and 2, 6, 24, or 48 hours after injection. Sera were tested for the presence of antibodies that bound to whole *T. spiralis* muscle-stage larvae in an enzyme-linked immunosorbent assay (ELISA). The ELISA titer was equal to the reciprocal of the dilution at which ELISA activity fell to five times the level of a normal rat serum diluted 1:50. Serum samples from pups were pooled for all time points—two samples at 0, 2, 6, and 24 hours and eight at 48 hours. Dams injected with 25 mg of normal rat Ig had no antibody to *T. spiralis* in their own or their pups' serum at any time. Data from a representative dam and her pups are graphed. Symbols: (x) serum from dam infused with antibody to *T. spiralis* and (○) serum from pups suckling dam infused with antibody to *T. spiralis*.

manifested in infant rats suckling an immune dam: many of the ingested *T. spiralis* were bound in mucus, and those that penetrated the intestinal epithelium were relatively immobile.

The relative importance of immunity to *T. spiralis* which is acquired in utero versus that conferred in colostrum or milk was determined by exchanging pups between immune and nonimmune dams at intervals after whelping (Fig. 2). Pups born to immune dams and fostered by a normal dam beginning on the first or third day of life were vulnerable to infection when challenged at 14 to 16 days. Evidently any immunity transferred in utero or in colostrum dissipates within 2 weeks. When pups were shifted from immune to nonimmune dams 1 day before challenge the immunity transferred in milk persisted for at least 24 hours. Pups shifted from nonimmune to immune dams were protected after fostering for only 24 hours.

We next confirmed an observation reported by Culbertson (*4*), namely that normal rat pups can be protected against *T. spiralis* by an intraperitoneal injection of specific serum from immune animals. We then used $(NH_4)_2SO_4$ precipitation to prepare a fraction of serum from immune animals which was rich in immunoglobulins (mainly IgG). This serum protected pups when injected intraperitoneally or given orally (Table 1). The protection was equivalent to that provided by an infected dam. Absorption of serum Ig with antiserum to rat IgG resulted in a significant loss of protection, while absorption with antiserum to rat IgA or IgM had no obvious effect. Therefore it is significant that antibodies to *T. spiralis* can be passaged serially through a lactating dam to her suckling young. This was demonstrated by infusing Ig from immune donors into lactating but otherwise normal rats. Antibodies originally present in the transferred material appeared in the blood of suckling pups after 24 hours (Fig. 3).

Antibodies probably mediate the immunity to *T. spiralis* that is transferred passively from a specifically immunized rat dam to her suckling young. Immunoglobulins G and A are the major antibody classes in rat milk, and they are present in similar amounts (*5*). That IgG is protective was revealed in the finding that IgG-rich serum (rat serum contains relatively little IgA) from immune donors protected newborn rats when ingested or injected intraperitoneally. The protective action of such antibodies may be attributable to the ready transport of serum antibodies to *T. spiralis* from the dam's blood into her milk and thence into the blood of suckling pups. Whether milk antibodies of other isotypes also protect remains an open question.

Our failure to diminish the protective capacity of specific serum antibody by adsorbing it to antibody to IgA does not mean that IgA is not involved in the mediation of rapid expulsion. Milk IgA may be qualitatively as well as quantitatively different from that in serum. The idea that IgG and IgA both mediate protection is appealing in light of (i) the capacity of IgA to protect mucosal surfaces, (ii) the association of *T. spiralis* larvae with the intestinal mucus of immune pups, and (iii) the ability of IgG to be transported readily across the intestinal epithelium of suckling rats (*6*) into the niche where the parasite lodges and is immobilized in immune rats. Mucus-associated muscle-stage larvae have been reported by others (*7, 8*) studying rapid expulsion in adult rats. Our experiments show that immunity to *T. spiralis* in neonates is passively transferred by antibodies, does not require previous intestinal exposure to the parasite, and is remarkably similar to rapid expulsion in adult rats.

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3 April 1984; accepted 11 July 1984

Interruption of the Mammillothalamic Tract Prevents Seizures in Guinea Pigs

Abstract. *Interruption of the connection between the mammillary bodies and the anterior nucleus of the thalamus in guinea pigs, by discrete bilateral electrolytic lesions of the mammillothalamic tract, resulted in essentially complete protection from the behavioral and electroencephalographic convulsant action and lethal effect of pentylenetetrazol. This result demonstrates that the mammillary bodies and their rostral efferent connections are important for the propagation and perhaps initiation of generalized seizures.*

The concept that subcortical regions in the mammalian brain propagate and perhaps initiate generalized seizures has been part of the biomedical literature for more than four decades (*1*). Several subcortical structures including various thalamic nuclei (*2*), the reticular formation (*3*), and, most recently, the substantia nigra (*4, 5*) have been suggested to play important roles in processes responsible for mediating generalized seizures.

We recently observed that guinea pigs infused with a combination of the convulsant drug pentylenetetrazol (PTZ) and the anticonvulsant drug ethosuximide, so that a prolonged state of minimal seizure activity was produced, had selective metabolic activation of the mammillary bodies, the mammillothalamic tracts, the anterior nuclei of the thalamus, and the ventral tegmental nuclei (*6*). This observation suggested that the mammillary bodies and their connections may be involved in the convulsant actions of PTZ. We now present evidence that supports this hypothesis and demonstrates that the pathway between the mammillary bodies and the anterior thalamus (mammillothalamic tracts) is important for the expression of experimental generalized seizures.

We examined the effects of interrupting the mammillothalamic tracts on the convulsant and lethal actions of PTZ. The mammillothalamic tracts were destroyed by stereotaxic electrocoagulation, and lesions were confirmed histologically. Clinical seizures following administration of various doses of PTZ were evaluated by a scoring system designed to rank the severity of clonic convulsant activity. The effect of PTZ on electroencephalographic (EEG) activity was also examined in paralyzed and ventilated animals. In these experiments, the duration of EEG seizure discharges in animals with lesions during a 1-hour experimental period was compared with that in control animals after injection of 150 mg of PTZ per kilogram of body weight; this supramaximal dose, in control animals, resulted in prolonged electrical seizures.

Guinea pigs (250 to 300 g), anesthe-