receptor α chain over a range of 34 amino acids, 21% homology over the entire 99–amino acid stretch with the *gag* polyprotein of the human immunodeficiency virus (HIV), about 24% homology (over a 62–amino acid range) with the genome polyprotein of poliovirus types 1 and 3, and 29% homology with the human *fos* transforming protein over a range of 31 amino acids, the significance of which, if any, remains to be established.
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41. We thank S. J. O'Brien for discussion and for providing somatic cell hybrid panels, D. Watson for the Hu-*ets* 2 cDNA clone, L. Lee for synthesizing oligonucleotide primers, K. McNitt and G. Smythers for assistance with the computer analysis, S. L. Chen for technical assistance, and K. Cannon for typing the manuscript.

9 March 1987; accepted 12 June 1987

Naturally Acquired Antibodies to Sporozoites Do Not Prevent Malaria: Vaccine Development Implications

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The first human vaccines against the malaria parasite have been designed to elicit antibodies to the circumsporozoite protein of *Plasmodium falciparum*. However, it is not known whether any level of naturally acquired antibodies to the circumsporozoite protein can predict resistance to *Plasmodium falciparum* malaria. In this study, 83 adults in a malaria-endemic region of Kenya were tested for circumsporozoite antibodies and then treated for malaria. They were monitored for the development of new malaria infections for 98 days. Antibody levels, as determined by four assays in vitro, were indistinguishable between the 60 individuals who did and the 23 who did not develop parasitemia during follow-up, and there was no apparent relation between day of onset of parasitemia and level of antibodies to circumsporozoite protein. Unless immunization with sporozoite vaccines induces antibodies that are quantitatively or qualitatively superior to the circumsporozoite antibodies in these adults, it is unlikely that such antibodies will prevent infection in areas with as intense malaria transmission as western Kenya.

WHEN A FEMALE ANOPHELINE mosquito carrying malaria sporozoites in her salivary gland feeds on a human, sporozoites pass into the bloodstream. Some of these sporozoites are transported to the liver, where they invade hepatocytes, initiating the cycle that culminates in malarial disease.

Sporozoites are covered by a polypeptide called the circumsporozoite (CS) protein. The CS protein of *Plasmodium falciparum* contains a large, immunodominant central domain consisting of 41 tandemly repeated tetrapeptides (37 Asn-Ala-Asn-Pro and 4 Asn-Val-Asp-Pro) (*1*). Development of human subunit sporozoite vaccines has been based on the hypothesis that antibodies to the repeat region of the *P. falciparum* CS protein will prevent sporozoite infection of hepatocytes and the subsequent development of malaria (*1–4*). The primary support for this hypothesis came from murine studies that showed that passive transfer of monoclonal antibodies to *P. berghei* sporozoites and polyclonal antibodies to synthetic peptides from the *P. berghei* repeat region protected mice against sporozoite challenge (*5, 6*). In a recent clinical trial of a recombinant DNA-produced malaria vaccine, human volunteers with vaccine-induced antibodies to the repeat region of the *P. falciparum* CS protein were challenged by the bite of five infected mosquitoes. The individual with the highest level of antibodies was protected against malaria infection, and two individuals with lower levels of antibodies had a delayed onset of parasitemia compared to controls (*7*). This study confirmed the hypothesis that humans can be protected from sporozoite infection by immunization with a subunit vaccine, and strongly suggested that the protection was mediated by antibodies.

Epidemiologic surveys in malaria-endemic areas have repeatedly shown that the prevalence of malaria decreases with increasing age, indicating that adults develop some protective immunity. Since most adults in such areas have antibodies to sporozoites (*8*), including antibodies to the CS protein repeat region (*9*), these antibodies have been considered to be possible mediators of protective immunity. In a retrospective study of sera from Indonesians living in a malaria-endemic area, the age-specific prevalence and titers of antibodies to the CS protein were inversely correlated with the age-specific prevalence of *P. falciparum* malaria. Affinity-purified antibodies to the repeat region of the CS protein derived from these sera mediated the circumsporozoite precipitation (CSP) reaction and inhibited sporozoite invasion of hepatoma cells (ISI) (*9*). These results were interpreted as supporting the hypothesis that antibodies to the CS protein repeat region protect against sporozoite infection. We now report the results of a prospective study designed to determine whether the level of naturally acquired antibodies to the CS protein would be predictive of protection against *P. falciparum* infection during a 98-day period when the rate of malaria transmission was high.

The study was conducted in Saradidi, western Kenya. In April 1986, 4 weeks prior to the peak malaria transmission season (*10*), 93 adult, male, lifelong residents of Saradidi volunteered for the study. Blood samples for serum and malaria smears were obtained from each volunteer, each of whom was then treated with a single dose of three tablets of pyrimethamine/sulfadoxine (Fansidar, Roche), followed by 100 mg of doxycycline twice daily for 7 days. On the basis of local parasite susceptibility to Fansidar alone, radical cures were expected for all volunteers. The home of each volunteer was visited daily for the next 98 days and blood smears were obtained on days 7, 14, 28, 42, 56, 70, 84, 98, and on any day a volunteer complained of illness. If malaria parasites

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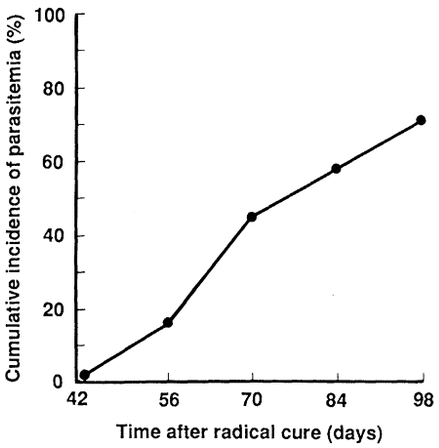

Fig. 1. Cumulative incidence of *P. falciparum* infections from day 42 through day 98. Eighty-three Kenyan adult males were treated with three tablets of Fansidar on day 0 and 100 mg of doxycycline twice a day on days 1 to 7. Each volunteer was visited daily for the next 98 days. Malaria smears were made on days 7 and 14 and every 2 weeks thereafter. A malaria thick smear was considered negative after two microscopists read 200 oil immersion fields (×1000).

were identified the volunteer was treated with amodiaquine. Venous blood for serum was obtained again on day 98.

Eighty-two of the volunteers aged 20 to 45 years and one aged 63 years completed the study (*11*). Thirty-two of the 83 (39%) had *P. falciparum* parasitemia on day 0. None was positive on days 7, 14, or 28 (*12*). Between days 42 and 98 there was a gradual increase in the cumulative proportion with parasitemia (Fig. 1). By day 98, 60 (72%) had become positive (*13*).

Serum samples obtained before treatment on day 0 from all 83 volunteers were tested by enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to the *P. falciparum* CS protein repeat region (*14*), by the indirect fluorescent antibody test (IFAT) for IgG antibodies to *P. falciparum* sporozoites (*8, 9*), for CSP activity (*15*), and for ISI activity (*16*). Mean antibody levels detected by the four assays (*17*) were not significantly different between the 32 individuals with *P. falciparum* parasitemia on day 0 and the 51 who did not have parasitemia. The results were also not significantly different between the 60 subjects who developed *P. falciparum* malaria from day 42 to 98 after radical cure and the 23 subjects who did not (Table 1). By ELISA, 96%, 73%, and 52% of volunteers had IgG antibodies to CS protein repeat epitopes at serum dilutions of 1:100, 1:400, and 1:1600, respectively, and 39% had IgM antibodies at a serum dilution of 1:50. Analyses by titer or antibody class did not reveal significant differences between infected and noninfected groups. Table 2 shows the relation between the day parasitemia was first detected after radical cure and the results of antibody assays in the four individuals with the highest IgG antibodies detected by ELISA, the highest scores in the ISI test, or the highest CSP scores. These tests did not predict resistance to malaria. Nor was the presence of CS antibodies of a specific IgG subclass (*18*) associated with resistance to malaria (Fig. 2).

To determine if higher levels of CS antibodies were associated with a delay in onset of parasitemia we used linear regression analyses. There was no statistically significant positive linear relation between level of antibodies by any of the assays and day of parasitemia. There were also no statistically significant differences in mean levels of antibodies in the group that was not infected by day 98 compared with the subgroups first infected on days 42 or 56, 70, 84, or 98.

Although the study was designed to last for 98 days, 19 of the 23 individuals who were not infected on day 98 had blood drawn on day 98 and were followed for an additional 28 days. Six had *P. falciparum* parasitemia on day 112 and three others on day 126. Antibody levels (ELISA) in sera obtained on day 98 were similar in the nine individuals who did and the ten individuals who did not develop parasitemia during the subsequent 28 days (mean ± SD absorbance at 1:100, 0.43 ± 0.307 and 0.46 ± 0.237, respectively).

To determine whether the intense exposure to sporozoites during the study period boosted antibodies to CS protein, we tested day 0 and day 98 sera from 75 subjects by ELISA for antibodies to $R32tet_{32}$ (*14*). The absorbance of sera diluted 1:100 increased from day 0 to day 98 in 74% of sera, as did mean absorbance for the group as a whole (0.39 ± 0.317 to 0.52 ± 0.352, $P \leq 0.0005$, paired t test). The increase in antibodies was similar among individuals who did and did not become infected during follow-up.

This study was undertaken because it was assumed that antibodies to CS protein that developed in individuals naturally exposed to sporozoites would be qualitatively similar to those elicited by immunization with purified CS proteins such as $R32tet_{32}$. However, since it is possible that the affinity or avidity of antibodies elicited by immunization with a purified protein might be different from that of antibodies elicited by repeated exposure to sporozoites, we have begun comparing the serum with the highest ISI-50 (see Table 2) from this study with the serum from the individual who was protected against sporozoite-induced malaria after immunization with $R32tet_{32}$ (*7*). Thus far we have found no differences between the immunoglobulin fractions of these sera in regard to ELISA and IFAT activity (*19*).

This study shows that levels of naturally acquired antibodies to the CS protein of *P. falciparum* that are at least as high as those induced by the first subunit malaria sporozoite vaccine (*7*) do not protect against infection with *P. falciparum* during a 98-day period of intense malaria transmission. The bite of only one infected mosquito can cause infection (*20*), and the sporozoite exposure of individuals in this study, each of whom received an average of 90 bites from infected mosquitoes during the 98-day study period (*10*), represents an enormous infectious challenge. It is possible that under conditions of less exposure, such as occur in Indonesia (*9*), such antibodies may play a more significant role in preventing infection than they did in this study.

The term semi-immune is often used by malariologists to describe adults from malaria-endemic areas, who are less susceptible to malaria than are children. The adults in this study had lifelong, intense exposure to malaria, yet 72% had *P. falciparum* infections 42 to 98 days after radical cure of infection. Despite this high attack rate, a parallel study carried out simultaneously in the same village in children aged 6 months to 5 years (*10*) indicated that the adults were less susceptible to malaria than were the children. Fifty-seven of 62 children (92%) developed *P. falciparum* parasitemia within 56 days

Table 1. Comparison of sporozoite antibodies in the group of 60 Kenyan adults that became infected and the group of 23 who did not become infected during 98 days. Sera obtained on study day 0 were tested at a 1:50 serum dilution by ELISA for IgG antibodies to R32LR (*14*), by IFAT for IgG antibodies to *P. falciparum* sporozoites (*8, 9*), for circumsporozoite precipitation (CSP) at a 1:2 serum dilution (*15*), and for inhibition of sporozoite invasion of hepatoma cells (ISI) at a 1:20 serum dilution (*16*). The results of the assays were similar in the infected and noninfected groups ($P > 0.05$). Results are expressed as mean ± SD.

Volunteers	ELISA IgG*	ISI (%)	Samples with >75% ISI (%)	CSP score	IFAT IgG ($-\log_{10}$ titer)
Infected ($N = 60$)	0.53 ± 0.48	67.5 ± 17.6	43	45.2 ± 13.5	2.46 ± 0.42
Not infected ($N = 23$)	0.55 ± 0.37	66.9 ± 17.4	39	36.8 ± 20.1	2.48 ± 0.29

*Measured as absorbance at 414 nm.

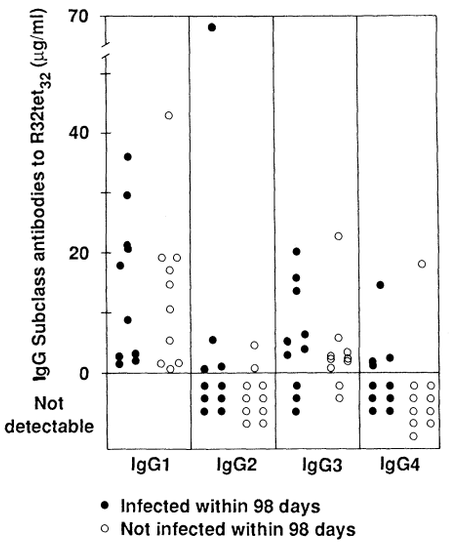

Fig. 2. Immunoglobulin G antibodies (subclasses 1 to 4) to $R32tet_{32}$ in sera from ten individuals who did (●) and ten individuals who did not (○) develop parasitemia within 98 days. Within the two groups, individuals were selected to include the five subjects with the highest levels of IgG antibodies (ELISA) and the five subjects with the lowest level of IgG antibodies. The concentrations of specific antibody subclasses are indicated (*18*).

after radical cure and 100% by day 84, whereas only 13 of 83 adults (16%) were positive by day 56 and 58% by day 84. Since there was no indication that adults had less exposure to infected mosquitoes than did children, the data suggest that some adults had acquired protective immunity.

At present there is direct evidence only for naturally acquired protective immunity against the blood stages of *P. falciparum* (*21*). Immunity against the blood stages of the parasite may account for the decreased susceptibility to malaria observed in the adults. However, mice and humans can be protected against sporozoite-induced malaria by immunization with irradiated sporozoites (*22–26*). This immunity is not directed against blood stages of the parasite (*24, 27*), but against sporozoites or exoerythrocytic parasites in the liver (pre-erythrocytic stages). This immunity can also be elicited by immunization with normal sporozoites in mice treated with chloroquine to prevent erythrocytic infection after maturation of exoerythrocytic parasites (*28*). It is possible that repeated infection of humans with normal sporozoites induces a similar immune response. Antibodies not detected in our assays may be important in this response, and could be directed against CS protein epitopes outside the repeat region (*29*), against an assembled topographic site on the CS protein not present conformationally on the *Escherichia coli*–derived protein used as antigen in the ELISA tests, or against sporozoite epitopes not present in the CS protein. However, the results of the IFA, CSP, and ISI tests, which use intact sporozoites as target antigens, do not support this interpretation. It is more likely that if protective immunity against pre-erythrocytic stages develops in humans under conditions of intense malaria exposure, the primary mechanisms are the same as those that protect mice immunized with sporozoites, and are largely independent of antibodies to sporozoites (*6*). Further understanding of these mechanisms, and the development of vaccines capable of eliciting them, will require identification of the antigens that elicit these responses, and the cells, cytokines, or other cellular products that mediate this protective immunity.

Table 2. The relation between the day when malaria parasites were first detected in the blood and the level of antibodies to sporozoites as measured by ELISA, ISI, CSP, and IFAT. Sera from the four individuals with the highest level of IgG antibody to R32LR, the four with the highest CSP score, and the four with the highest percent ISI were selected. ELISA data show mean absorbance of triplicate sera at a dilution of 1:50; ISI results show the percent inhibition at a serum dilution of 1:20; CSP results show the CSP score (*15*) at a serum dilution of 1:2 and the last reciprocal serum dilution at which the CSP score was ≥8; and IFAT results show the last reciprocal dilution at which there was fluorescence. Neg, negative.

Day parasites found in blood	ELISA 1:50	ISI (%)	CSP score	CSP titer	IFA titer
98	**2.245**	86.3	62	128	1280
70	**1.677**	59.9	36	128	320
84	**1.619**	51.7	34	8	320
98	**1.342**	67.3	60	32	1280
70	0.351	**91.8**	28	8	320
56*	1.270	**91.5**	46	128	1280
Neg†	1.269	**91.1**	4	Neg	1280
42	0.159	**90.2**	48	2	320
98	0.229	69.1	**80**	32	320
Neg	0.326	79.7	**76**	32	320
56	0.059	74.1	**72**	8	80
70	0.829	76.1	**70**	32	1280

*This serum had the highest ISI-50 (*16*); the last dilution at which the serum inhibited sporozoite invasion of hepatoma cells ≥50%. †This individual developed parasitemia on day 112.

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10. C. N. Oster *et al.*, Abstract No. 349, Annual Meeting of the American Society of Tropical Medicine and Hygiene, December 1986 (in preparation). The mean *P. falciparum* entomologic inoculation rate (EIR) was 0.37 potentially infective bites per human per night during the 2 weeks prior to and the 4 weeks after initiation of the study, and 1.2 during weeks 5 to 14.
11. It was predicted that the attack rate during follow-up would be 50%, and that serum samples from 10% of volunteers who became infected and 50% of those who did not become infected would inhibit sporozoite invasion (ISI) >75%. On the basis of equal-sized groups, a total of 50 volunteers was required to show that this difference in ISI was significant ($\alpha = 0.05$, one-tailed, $\beta = 0.10$). Because of uncertainty in prediction of attack rate and compliance a sample of 90 to 100 individuals was chosen. Ninety-three individuals gave informed consent and were entered. Ten dropped out voluntarily during follow-up.
12. A slide was considered negative when 200 fields of a thick smear were read at ×1000 by two microscopists during the 2 weeks after it was taken. The study design called for any individual who was positive on day 28 to be excluded from the study, since recrudescence secondary to resistance could not be distinguished from reinfection. Review of the blood films after the study was concluded revealed that an individual who was positive for parasites on day 0 and initially considered to be negative on day 28, actually had fewer than ten parasites per microliter of blood on day 28. Since this individual was not treated and had negative blood films on days 42, 56, 70, 84, 98, 112, and 126, he has been included in the no reinfection group.
13. A definition of resistance to the combination of Fansidar and doxycycline has not been established. Resistance to many antimalarials, including Fansidar, is generally defined as recurrence of infection within 28 days of treatment. Blood smears from all individuals were negative for parasites on days 7 and 14. One individual (*12*) had transient parasitemia on day 28 which cleared without treatment and did not recur, and the two volunteers found to be positive for *P. falciparum* on day 42 were negative on day 0. It is highly unusual for resistant parasites to become detectable for the first time 42 or more days after treatment with Fansidar. Thus, the infections detected almost certainly represented new infections, not recurrences of old infections.
14. Antigens for the ELISAs were $R32tet_{32}$ (*3*): Met-Asn - Pro[(Asn - Ala - Asn - Pro)$_{15}$Asn - Val - Asp - Pro]$_2$tet$_{32}$; or R32LR: Met-Asn-Pro[(Asn-Ala-Asn-Pro)$_{15}$Asn-Val-Asp-Pro]$_2$Leu-Arg [S. L. Hoffman *et al.*, *Exp. Parasitol.*, in press]. Both were recombinant fusion proteins produced in *Escherichia coli* and consisted of 32 tetrapeptide repeats (R32) derived from the CS protein of *P. falciparum* fused to either 32 ($R32tet_{32}$) or two (R32LR) amino acids derived from the tetracycline resistance region of the plasmid. ELISA was performed in 96-well, flat bottom Immulon II plates (Dynatech Laboratories) as described (*9*), except that 0.4% boiled casein instead of bovine serum albumin was used for blocking. Color development was measured at 414 nm by an ELISA reader (Titertek Multiskan MCC). Because of variability in absorbance of sera in wells without antigen, the absorbance of a serum at any dilution was calculated as the mean absorbance of triplicate wells with antigen minus the mean absorbance of triplicate wells without antigen. At each dilution, a serum was considered to have detectable antibody to the antigen if the mean absorbance was greater than the mean + 3 SD of 14 to 16 control sera from American residents of Washington, D.C. The mean + 3 SD of control sera at dilutions of 1:50, 1:100, 1:400, and 1:1600 for IgG antibody to R32LR were 0.109, 0.024, 0.040, and 0.033, respectively, and at 1:50 for IgM antibody was 0.048.

15. Circumsporozoite precipitation (CSP) tests [J. Vanderberg, R. Nussenzweig, H. Most, *Milit. Med.* **134**, 1183 (1969)] were performed as described (*9*). Twenty-five sporozoites were examined and the number with granular precipitates on the surface (2+ reaction) or with a long threadline filament at one end (4+ reaction) determined. The CSP score was the number of sporozoites with 2+ reactions multiplied by 2, plus the number of sporozoites with 4+ reactions multiplied by 4. A score of 8 was considered positive.
16. Inhibition of sporozoite invasion of hepatoma cells (ISI) [M. R. Hollingdale, E. H. Nardin, S. Tharavanij, A. L. Schwartz, R. S. Nussenzweig, *J. Immunol.* **132**, 909 (1984)] was performed as described (*9*). Assays were performed in triplicate at a serum dilution of 1:20. The percent ISI = [100][1 − (number of sporozoites that entered in the presence of test serum) ÷ (number of sporozoites that entered in the presence of control serum)]. The four sera with the highest ISI (Table 2) were serially diluted to determine the last dilution at which sporozoite invasion was inhibited ≥50% (ISI-50). The ISI-50 was >1:300 but <1:1000 in two sera, and <1:300 in the other two.
17. Although monoclonal antibodies to CS protein repeat epitopes react in the four assays, the target antigens for the ELISA (purified protein from the repeat region of the CS protein), IFAT (air-dried sporozoites), and ISI and CSP (live sporozoites) are different, and these assays may measure antibodies to different epitopes. In fact, when sera from all 83 individuals were compared, there was a significant correlation only between the results of the IgG IFAT and IgG ELISA ($r = 0.45$, 95% confidence interval = 0.27–0.61, $P < 0.00001$) and the IgG IFAT and ISI ($r = 0.30$, 95% confidence interval = 0.11 to 0.48, $P = 0.0028$).
18. Concentrations of IgG subclass antibodies to $R32tet_{32}$ were determined as described (*9*). Dilutions of purified myeloma proteins of each of the four subclasses were used to establish standard curves. At the 1:50 serum dilution used in these assays, the minimum detectable concentrations of IgG1, IgG2, IgG3, and IgG4 antibodies to $R32tet_{32}$ were 0.75, 0.95, 0.95, and 1.6 μg/ml, respectively. (Myeloma proteins and antibodies to subclasses were provided by R. Wistar, Jr., and C. Cole.)
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2 April 1987; accepted 23 June 1987

Astrocytes Block Axonal Regeneration in Mammals by Activating the Physiological Stop Pathway

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Regenerating sensory axons in the dorsal roots of adult mammals are stopped at the junction between the root and spinal cord by reactive astrocytes. Do these cells stop axonal elongation by activating the physiological mechanisms that normally operate to stop axons during development, or do they physically obstruct the elongating axons? In order to distinguish these possibilities, the cytology of the axon tips of regenerating axons that were stopped by astrocytes was compared with the axon tips that were physically obstructed at a cul-de-sac produced by ligating a peripheral nerve. The terminals of the physically obstructed axon tips were distended with neurofilaments and other axonally transported structures that had accumulated when the axons stopped elongating. By contrast, neurofilaments did not accumulate in the tips of regenerating axons that were stopped by spinal cord astrocytes at the dorsal root transitional zone. These axo-glial terminals resembled the terminals that axons make on target neurons during normal development. On the basis of these observations, astrocytes appear to stop axons from regenerating in the mammalian spinal cord by activating the physiological stop pathway that is built into the axon and that normally operates when axons form stable terminals on target cells.

IN THE ADULT MAMMALIAN CENTRAL nervous system (CNS), regenerating axons typically grow relatively short distances—less than 1 mm. The restriction of axonal elongation in the adult CNS is not a limitation of the intrinsic capacity of axons to elongate (*1, 2*); but rather, cellular elements of the CNS, particularly astrocytes, appear to restrict the elongation of axons (*3, 4*).

The effects of mature mammalian astrocytes on axonal elongation can be studied at the transitional zone between the dorsal root and the dorsal root entry zone of the spinal cord (*1, 5–8*). By transecting the axons in the dorsal root, it is possible to study their regeneration from the root into the spinal cord without directly injuring the cord. Trauma to the cord results in the formation of a connective tissue scar that contains fibroblasts and a dense collagenous matrix (*1, 4*), which has been shown to prevent axonal elongation (*1, 9*). In the dorsal root regeneration model, there is no connective tissue scar and axonal growth in a purely astroglial environment can be studied.

Regenerating axons in the dorsal root are surrounded by a substrate that contains Schwann cells and their basal laminae. This milieu is similar to that in a peripheral nerve and supports the active elongation of regenerating axons, which grow until they reach the dorsal root transitional zone. At this interface between root and spinal cord, the regenerating axon tips encounter a substrate that consists almost entirely of reactive astrocytic processes. Here, among these processes, many of the growth cones stop and form stationary axon terminals that remain in place for a year or more (*6–8*). These observations suggest that mature astrocytes can stop the intrinsic tendency of axons to elongate through their effects on the axon tip.

By what mechanisms do astrocytes stop axonal elongation? Normally, a physiological sequence is activated in the axon tip when it makes synaptic contact with an appropriate postsynaptic neuron in the CNS (*10–12*). One important part of this physiological pathway is the disassembly of cytoskeletal polymers (neurofilaments and microtubules) that are continuously transported into the stationary axon tip. Specifically, proteases that degrade the neurofilaments are selectively activated in the presynaptic terminal (*11–14*); if these proteases are inhibited by the protease inhibitor leupeptin, neurofilaments accumulate in the axon terminal (*15*).

Axons can also be stopped from elongating without fully activating the physiological pathways that degrade the neurofilaments. If axons are physically obstructed from elongating by being forced to grow into a cul-de-sac that is produced by tightly ligating a peripheral nerve, the trapped axon tips fill with neurofilaments and other axonally transported structures (*13, 16*). This

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