with priming for help to CS–vaccinia virus.

We next constructed a synthetic immunogen containing T-cell and B-cell sites derived from the CS molecule. We prepared Th2R-NP(NANP)$_5$NA (*18*) and immunized B10.BR and B10.A(4R) mice with either this or NP(NANP)$_5$NA and then measured their antibody production in response to (NANP)$_n$. Both strains responded to the conjugate peptide but not to NP(NANP)$_5$NA alone (Fig. 5). In the native molecule, the NANP repeating unit is located between Th2R and the amino terminus, whereas in the synthetic construct, NP(NANP)$_5$NA was located at the carboxyl terminal end of Th2R. This change in orientation did not affect the ability of the T-cell site to generate carrier-specific help for the responsive B cells. Thus a synthetic immunogen was designed and constructed containing a T-cell site and a B-cell site, both of parasite origin.

While the CS protein appears to contain few major epitopes capable of stimulating T cells to help B-cell response against (NANP)$_n$, it may contain other epitopes responsible for T-cell proliferation, which may be important for antibody-independent cellular immunity. As mentioned, peptide 103 to 116 may represent such an epitope. As well, it may contain other sites recognized by cytotoxic T cells that may play a role in sporozoite immunity. While we have looked at helper T-cell epitopes that produce a B-cell response against (NANP)$_n$, other helper T-cell epitopes may be present that may preferentially help B cells of other specificities (*13, 14*); however, the specificity of the immunoglobulin antibody to (NANP)$_n$ is the one known to neutralize sporozoites. T-cell sites are required both for helper function in antibody production and for antibody-independent cellular immunity, both of which appear to be important in immunity to sporozoites (*5, 8*).

A vaccine to be used in endemic areas would rely on natural boosting from sporozoites. If natural antibody boosting by sporozoites is required, or if antibody-independent T-cell immunity is critical, a vaccine must contain parasite-derived T-cell epitopes. Natural boosting by sporozoites would maintain a high antibody titer, known to be necessary for antibody-mediated protection, as well as maintain T cells in an activated state; however, if T-cell sites were limited on the CS molecule, a vaccine reliant on natural boosting might be ineffective in some people. The more such T-cell sites that are incorporated in a vaccine, the more this problem should be minimized. The approach outlined here should be useful in the rational design of synthetic or recombinant fragment vaccines.

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11. A description of the CS–vaccinia virus will be presented elsewhere. While it may have been theoretically possible to perform some of these experiments with sporozoites instead of virus, it is difficult to produce sporozoites from the *P. falciparum* strain (7G8) whose CS protein has been sequenced.
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18. The Th2R-NP(NANP)$_5$NA conjugate was prepared as follows: 5.7 mg of *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) (Pierce Chemicals) in 380 μl of dimethylformamide was added dropwise to 15 mg of NP(NANP)$_5$NA in 1.5 ml of 10 m*M* $NaPO_4$, *p*H 7.0. The mixture was stirred for 30 minutes at room temperature and then the *m*-maleimidobenzoyl peptide (MB-peptide) was separated from the unreacted MBS by desalting on a column of Sephadex G-25 in 50 m*M* PO_4, *p*H 6.0. The resulting MB-peptide pool was mixed with 15 mg of the Th2R peptide in 15 ml of phosphate-buffered saline (PBS), *p*H 7.2, and the mixture stirred at room temperature for 3 hours. The final Th2R-NP(NANP)$_5$NA conjugate was desalted on a column of Bio-Gel P-2 (Bio-Rad) and analyzed by reversed-phase high-performance liquid chromatography and its amino acid composition was determined.
19. Immulon-1 microtiter plates (Dynatek, Alexandria, VA) were coated with R32tet$_{32}$ (*3*), 100 μl per well at a concentration of 2 μg/ml. The plates were washed and the sera diluted in PBS, *p*H 7.4, containing 1% bovine serum albumin (BSA) and 0.05% Tween 20. After incubation for 1 hour at 37°C followed by washings, horseradish peroxidase–conjugated goat antiserum to mouse immunoglobulin was added. The plates were incubated again for 1 hour at 37°C, washed, and substrate added. The substrate was 2,2′-azinodi(3-ethylbenzthiazoline-sulfonate). The reaction was stopped by the addition of 1.25% KI. Optical density was read at 414 nm with a flow Titertek Multiskan.

20. We thank Smith, Kline, and French Laboratories, Swedeland, PA, for providing R32tet$_{32}$; K. Cease, W. Weiss, W. Hockmeyer, S. Ozaki, S. Brett, and M. Kojima for discussions; D. H. Sachs and T. A. Waldmann for reading of the manuscript; D. H. Sachs for providing certain mouse strains; P. Spinella and D. Pombo for laboratory assistance; and W. Davis for editorial assistance. Partial support for MFG came from a Neil Hamilton Fairley Fellowship from the National Health and Medical Research Council (Australia) and a Fulbright Postdoctoral Award.

27 October 1986; accepted 26 January 1987

Apogeotropic Roots in an Amazon Rain Forest

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Roots of some tropical trees grow vertically upward on the stems of neighboring trees. Apogeotropic roots occur in 12 species across five families. These roots, originating as fine roots in the mineral soil, grow upward as fast as 5.6 centimeters in 72 hours. Apogeotropic root growth may be an adaptation to extremely low soil nutrient availability in Amazon forests. In these forests upward-growing roots obtain nutrients via the predictable pathway of precipitation that flows down along the stem. Apogeotropic roots form a nutrient cycling pathway in which nutrients are absorbed and transported directly from plant to plant, without entering the soil solution.

APOGEOTROPIC ROOTS GROW OUT of the soil and up tree stems. In a nutrient-poor Amazonian rain forest, they are an important phenomenon that constitutes an unusual and previously unreported nutrient cycling pathway. Roots of tropical trees in these forests are usually most abundant near the soil surface, although they sometimes develop into a "root mat" above the mineral soil surface (*1*). In some tropical forests, adventitious roots originating in the forest canopy absorb mineral nutrients from canopy detritus (*2*). Climbing roots, common in Amazon forests near San Carlos de Rio Negro, Venezuela (1°56′N, 67°03′W), originate in the mineral soil and the surface root mat, and sometimes grow >13 m up the outer surfaces of the bark of live trees. In a 0.1-ha area sampled in this study, all stems >4 cm dbh [diameter at breast height (137 cm)] hosted climbing roots morphologically indistinguishable from small-diameter subterranean roots. At least 12 species in Amazonia *tierra firme* (nonflooded) forest are capable of producing these apogeotropic roots. Individuals of one species, *Eperua purpurea* Bentham, send roots up their own stems; no climbing roots of other species are found on them. I propose that apogeotropic roots grow in response to a nutrient gradient caused by the flow of precipitation on the stem (stem flow) in these otherwise nutrient-scarce forests.

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Nutrient content of both soils and fallen litter in forests near San Carlos de Rio Negro are among the lowest reported worldwide for tropical forests (*3*). Calcium is particularly low in *tierra firme* soils and litter fall (*4*). Nutrients in precipitation are an especially important source of nutrients to plants in these forests. Nutrient scavenging, the process whereby nutrients in precipitation are absorbed in the canopy, has been demonstrated for this *tierra firme* forest, but stem flow is a relatively rich source of nutrients (*5*). Approximately 7% of annual precipitation at San Carlos reaches the forest floor as stem flow; calcium, magnesium, and potassium concentrations in this stem flow are less than those reported for temperate-zone forests, whereas nitrogen and phosphorus concentrations are approximately equivalent (*6*). However, high annual rainfall (>3500 mm $year^{-1}$) at San Carlos results in comparatively high total nutrient transfer via stem flow; the total nutrient transfer by stem flow (19% of throughfall) is greater than in most other forests (12%) (*7*). High average monthly rainfall (>200 mm $month^{-1}$ in the driest month) and consequent high relative humidity create conditions that allow root growth above the soil surface.

A survey of the root mat in *tierra firme* forests near San Carlos de Rio Negro revealed thicker root mats near tree stems than away from tree stems. This is similar to a pattern observed for trees elsewhere in the tropics (*8*). In forests near San Carlos de Rio Negro, however, roots from the root mat extend upward on the bark surface of all tree stems. These small roots (<2 mm in diameter) grow on exposed surfaces, within the fissures of bark, and beneath attached epiphytic mosses, ferns, and vines. I found no indication that roots growing on live stems penetrated the bark to the cambium.

A complete survey of the 95 stems greater than 4 cm dbh in a 500-m^2 subplot in *tierra firme* forest revealed one or more climbing roots on every stem and a significant (paired *t* test: $P < 0.0001$) increase [16.0 ± 1.6 cm (mean ± SEM)] in the depth of the root mat adjacent to stems. Mean depth of the root mat adjacent to stems was 36.1 ± 1.8 cm (± SEM) versus 20.1 ± 0.9 cm, for the mat measured 1 m from stems. The mean height of the uppermost root on the 95 measured stems was 152 ± 9 cm. Usually each stem supported more than one climbing root; the mean number of climbing roots per stem was 2.6 (SD, 2.3; range, 1 to 15). In one case an individual (*Caryocar* sp.) supported 15 climbing roots, one of which was from a nearby *Swartzia* sp. stem that grew 13.4 m upward into the subcanopy branches. At this height, the root ramified extensively in a large decomposing branch. Repeated observation revealed that during the wet season, abundant, rapidly emerging root tips were present.

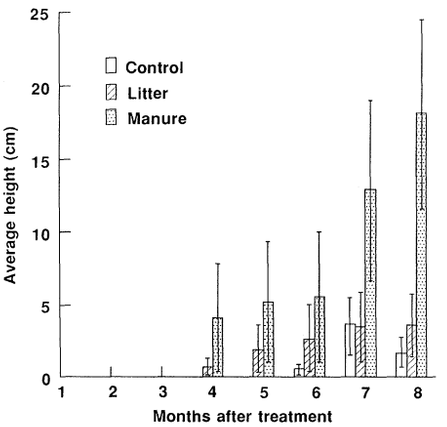

Fig. 1. Average heights (± standard error) of apogeotropic roots growing on artificial stems topped with cylinders empty (control) or filled with forest floor litter or manure ($n = 8$ per treatment).

In order to trace climbing roots to their source stems, I excavated every climbing root (growing up stems >1 cm dbh) to its stem of origin within a 100-m^2 plot located randomly in *tierra firme* forest. The climbing roots on almost all stems originated from a source stem of a different species. The sole exception was roots of *Eperua purpurea* Bentham (Caesalpiniaceae). Each stem of this species was occupied exclusively by roots originating underground from the same stem. Twelve of 50 stems (24%) representing 12 species were sources of climbing roots (Table 1). Of these 12 stems, 3 stems sent forth 60% of all climbing roots: *Eperua purpurea* 30%, *Brosimum utile* (H.B.K.) Pittier (Moraceae) 17%, and *Eperua leucantha* Bentham (Caesalpiniaceae) 13%. The mean absolute distance from a source stem to a support stem was 4.0 m (SD, 2.3 m; range, 0 to 14.3 m). The mean root length between source stems and support stems was 5.4 m (SD, 4.7 m; range, 0 to 20.7 m).

To test the hypothesis that stem flow contributes to root apogeotropism, 24 artificial tree stems (plastic pipes, 16 cm in diameter by 2 m in height) were placed upright in intact *tierra firme* forest. Three stiff nylon mesh (2 mm by 2 mm mesh opening) cylinders containing different mineral nutrient sources were attached to each simulated stem 50 cm above the soil surface. One-third of the cylinders contained cattle manure, one-third contained forest floor litter, and one-third were empty (control) cylinders. Attached to the upright stems in November, the cylinders were examined monthly for 8 months for climbing roots.

Within 4 months, climbing roots appeared on eight of the artificial stems with manure, two of those with litter and none of the control stems. After 7 months, roots had ascended two of the control stems. By 8 months, all artificial stems with manure supported ascending roots with an average height of 18.2 cm (Fig. 1). The average root heights on litter-baited and control artificial stems were 3.6 and 1.7 cm, respectively. The roots that ascended control stems did so at slow rates; the uppermost did not exceed 6.2 cm height.

To document short-term trends, I measured a subset of roots every 72 hours for a 12-day period 7 months after the initiation of the experiment. At that time, climbing roots were well established on artificial stems, but had not exceeded 1.5 m height. The average root height increment was 0.7 cm (SD, 1.2 cm; range, −0.7 to 5.6) per 72 hours. Occasional reduction in root height was caused by grazing or the death of root tips.

The physiological basis of root gravitropism is a topic of considerable research and debate (*9*). Calcium gradients play a key role in initiating root gravitropism: asymmetric application of calcium near the tips of roots causes curvature toward the calcium source in both decapped and intact roots (*10*). A calcium gradient may contribute to root apogeotropism in Amazonian forests. In the same *tierra firme* forest near San Carlos de Rio Negro, fine root growth was examined with mineral-salt "baits" buried within the root mat (*11*). Greatest root growth coincided with calcium sources. Canopy calcium leaching via stem flow could form a zone of

Table 1. Twelve species of tropical trees and plants found to exhibit root apogeotropism in a 100-m^2 study area of Amazon *tierra firme* forest near San Carlos de Rio Negro, Amazon Territory, Venezuela. It is likely that *tierra firme* forest species in addition to those listed are capable of producing apogeotropic roots.

Family and species
Caesalpiniaceae
Eperua purpurea Bentham
Eperua leucantha Bentham
Swartzia sp.
Moraceae
Brosimum utile (H.B.K.) Pittier
Brosimum sp.
Ficus sp.
Myristicaceae
Virola surinamensis (Rol.) Warb.
Chrysobalanaceae
Licania heteromorpha Bentham
Arecaceae
Jessenia bataua (Mart.) Burret
Araceae
Philodendron sp.
Other
Liana (not determined)
Liana (not determined)

calcium enrichment on tree stems during rain. This enrichment may be part of a general nutrient tropism caused by stem flow that would enhance root growth near the base of tree stems and that ultimately may cause root apogeotropism in these forests. This idea is supported by (i) increased depth of the root mat at the base of stems, (ii) increased rates of root growth with nutrient enrichments especially toward calcium sources, and (iii) the key role of calcium in initiating root apogeotropism.

Several intriguing questions remain concerning apogeotropic roots. The total number of species that produce these roots was not determined by this study. The mycorrhizal status of these roots is unknown. Apogeotropic roots of *Eperua purpurea* are abundant on stems of other species, but for unknown reasons, other species' roots are excluded from growing up stems of *Eperua purpurea*. Climbing roots have yet to be widely examined as a feature of nutrient cycling in tropical rain forests. There is anecdotal evidence, however, that roots in Asian tropical rain forests are capable of climbing nearby stems (*12*). I have observed apogeotropic roots in *Metrosideros* sp. forests in Hawaii Volcanoes National Park, Hawaii (19°31'N, 155°27'W), and in rain forests near La Selva Biological Station (10°25'N, 84°01'W) in Costa Rica in addition to those at San Carlos de Rio Negro.

Climbing roots have evolved in rain-forest tree species representing at least five families (Table 1). These roots appear to have evolved in environments with low soil nutrient availability, but where a reliable, relatively rich source of nutrients is available via stem flow. Species with climbing roots absorb stem flow nutrients before these nutrients enter the soil and become either generally available to the roots of all species or unavailable to plants because of leaching and strong adsorption on soil particles. Climbing roots form a nutrient cycling pathway in which nutrients entering tropical forests in the form of stem flow are absorbed and transported from one stem to another without entering the soil solution.

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25 September 1986; accepted 31 December 1986

Relationship Between the c-*myb* Locus and the 6q− Chromosomal Aberration in Leukemias and Lymphomas

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Deletions of the long arm of chromosome 6 (6q−) are frequently found in hematopoietic neoplasms, including acute lymphoblastic leukemias, non-Hodgkin lymphomas and (less frequently) myeloid leukemias. The c-*myb* proto-oncogene has been mapped to region 6q21–24, which suggests that it could be involved in the 6q− aberrations. By means of in situ chromosomal hybridization on cells from six hematopoietic malignancies, it was demonstrated that the c-*myb* locus is not deleted, but is retained on band q22, which is consistently bordered by the chromosomal breakpoints in both interstitial and terminal 6q− deletions. The deletion breakpoints were located at some distance from the *myb* locus since no rearrangement of c-*myb* sequences was found. In one case, however, amplification of the entire c-*myb* locus was detectable. Furthermore, in all cases tested that carry 6q− deletions, *myb* messenger RNA levels were significantly higher than in normal cells or in malignant cells matched for lineage and stage of differentiation but lacking the 6q− marker. These results indicate that 6q− deletions are accompanied by structural and functional alterations of the c-*myb* locus and that these alterations may be involved in the pathogenesis of leukemias and lymphomas.

NONRANDOM AND SPECIFIC CHROMOSOME aberrations have been described in several malignant disorders of animals and man (*1*). A role for these cytogenetic abnormalities in the pathogenesis of different tumors is supported by the findings that in some cases proto-oncogene loci are specifically involved in the chromosomal recombinations (*2*). This is the case for the c-*myc* and c-*abl* loci in the (8;14) and (9;22) translocations typical of undifferentiated B-cell lymphoma (UBL) and chronic myelogenous leukemia (CML), respectively (*3–4*).

A frequent, yet poorly characterized chromosomal abnormality is represented by an apparently heterogeneous group of deletions of part of the long arm of chromosome 6 (6q−), including both interstitial and terminal deletions with breakpoints at region 6q21–23. The 6q− abnormalities were originally reported as a relatively frequent observation in acute lymphoblastic leukemia (ALL) (*5*) and are included in the restricted catalog of chromosomal defects that can be found as single aberrations in a defined group of tumors (*6*). Recent studies involving large panels of cases have reported that 6q− deletions are found in 5 to 25% of ALL (*5, 7*), in 30% of non-Hodgkin lymphomas (NHL) (*8*), and, less frequently, in acute myelogenous leukemia (AML) and CML (*9*).

The c-*myb* proto-oncogene has been localized on chromosome 6 in the approximate region 6q21–24 (*10*). The possible relationship between the 6q− abnormality found in hematopoietic neoplasms and the c-*myb* gene is particularly intriguing, in view of the apparent hematopoiesis-specific expression of this gene. In fact, c-*myb* messenger RNA (mRNA) has primarily been found in hematopoietic cells, where relatively high levels are detectable in immature myeloid and lymphoid precursors (*11*). Expression of c-*myb* is induced in proliferating immature hematopoietic cells (*12*) and suppressed in terminally differentiated cells (*10, 13*), suggesting that this gene may be involved in the

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