contained in the 1.5-kb 3′ exon of this subgenomic message. Immediately 5′ to this exon is a second exon of about 140 nucleotides that spans the *pol–env* junction and provides the initiation codon for the *x* gene. The 5′ exon appears to be a leader sequence of about 135 nucleotides. It extends from the cap site to a splice donor site located in the stem of the potential hairpin loop that has been proposed for the HTLV-I and HTLV-II LTR (*12, 27, 28*). We believe this to be the entire structure of the HTLV-II *x* subgenomic message. These data are also consistent with the observations of Seiki *et al.* (*29*) regarding the structure of HTLV-I *x* mRNA.

The functional basis for this precise scheme of mRNA processing to generate the *x* mRNA is unclear. The only other example of retroviral RNA processing in which the virus contains a gene between *env* and the 3′ LTR is Rous sarcoma virus (*30, 31*). In this case, sequences from *env* are not required to generate the *src* gene product (*32, 33*). It is possible that this type of mRNA processing, which adds only four nucleotides of coding sequence to downstream *x* sequences for HTLV, is a primary regulatory mechanism for the production of the *x* product. The function of $p40^{xI}$ and $p37^{xII}$ is unknown. These proteins may be important in viral transcription (*34, 35*). Recent *x* gene mutagenesis studies in our laboratory indicate that the *x* protein increases the efficiency of HTLV transcription (*35a*). Regulation of the expression of the *x* protein may be important for viral transcription and replication as well as for the initiation and maintenance of cellular transformation.

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References and Notes

1. B. J. Poiesz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7415 (1980).
2. Y. Hinuma *et al.*, *ibid.* **78**, 6476 (1981).
3. W. A. Blattner *et al.*, *Int. J. Cancer* **30**, 257 (1982).
4. A. Saxon, R. H. Stevens, D. W. Golde, *Ann. Intern. Med.* **88**, 323 (1978).
5. V. S. Kalyanaraman, M. G. Sarngadharan, M. Robert-Guroff, I. Miyoshi, D. Blayney, D. Golde, R. C. Gallo, *Science* **218**, 571 (1982).
6. I. Miyoshi, I. Kubonishi, S. Yoshimoto, T. Akagi, Y. Ohtsuki, Y. Shiraishi, K. Nagata, Y. Hinuma, *Nature (London)* **294**, 770 (1981).
7. N. Yamamoto *et al.*, *Science* **217**, 737 (1982).
8. M. Popovic *et al.*, *ibid.* **219**, 856 (1983).
9. I. S. Y. Chen, S. G. Quan, D. W. Golde, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7006 (1983).
10. M. Seiki, R. Eddy, T. B. Show, M. Yoshida, *Nature (London)* **309**, 640 (1984).
11. J. C. Gasson, personal communication.
12. M. Seiki *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3618 (1983).
13. I. S. Y. Chen *et al.*, *Nature (London)* **305**, 502 (1983).
14. W. A. Haseltine *et al.*, *Science* **225**, 419 (1984).
15. K. Shimotohno *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 6657 (1984).
16. N. R. Rice *et al.*, *Virology* **138**, 82 (1984).
17. N. Sagata *et al.*, *EMBO J.* **3**, 3231 (1984).
18. W. Wachsman *et al.*, *Science* **226**, 177 (1984).
19. D. J. Slamon *et al.*, *ibid.*, p. 61; T. H. Lee *et al.*, *ibid.*, p. 57.
20. P. Mellon and P. H. Duesberg, *Nature (London)* **270**, 631 (1977).
21. B. Cordell *et al.*, *Cell* **15**, 79 (1978).
22. P. B. Hackett *et al.*, *J. Virol.* **41**, 527 (1982).
23. K. Shimotohno *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
24. H. M. Temin, *Cell* **28**, 3 (1982).
25. T. Yamamoto, B. de Crombrugghe, I. Pastan, *ibid.* **22**, 787 (1980).
26. E. W. Benz, Jr., *et al.*, *Nature (London)* **288**, 665 (1980).
27. J. Sodroski *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4617 (1984).
28. N. Sagata *et al.*, *ibid.*, p. 4741.
29. M. Seiki *et al.*, *Science* **228**, 1532 (1985).
30. S. R. Weiss, H. E. Varmus, J. M. Bishop, *Cell* **12**, 983 (1977).
31. N. Quintrell *et al.*, *J. Mol. Biol.* **143**, 363 (1980).
32. A. F. Purchio *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1567 (1978).
33. K. Beemon and T. Hunter, *J. Virol.* **28**, 551 (1978).
34. J. G. Sodroski, C. A. Rosen, W. A. Haseltine, *Science* **225**, 381 (1984).
35. C. A. Rosen, J. G. Sodroski, R. Kettman, A. Burny, W. A. Haseltine, *ibid.* **227**, 320 (1985).
35a. I. S. Y. Chen *et al.*, *ibid.*, in press.
36. A. J. Berk and P. A. Sharp, *Cell* **12**, 721 (1977).
37. R. Weaver and C. Weissman, *Nucleic Acids Res.* **7**, 1175 (1979).
38. SLB-I and SLB-II are cell lines derived by infection of normal human leukocytes with HTLV-I in vitro.
39. A. Saxon *et al.*, *J. Immunol.* **120**, 777 (1978).
40. The cDNA clone was selected by hybridization to an oligonucleotide probe, 5′-CAAATCC TAGGAAATGG-3′ (complementary to nucleotides 7215–7231) (*23*), which is downstream of the *x* splice-acceptor site, and by the lack of hybridization to an oligonucleotide probe, 5′-CTCCTCTCAGCCCATTTCC-3′, which spans the *x* splice acceptor sequences (nucleotides 7204–7222) (*23*). A third hybridization probe was an HTLV-II cDNA clone previously described and located within the *x* gene (*13*). Nucleic acid sequencing of this portion of the *x* cDNA clone was performed by the standard dideoxy technique.
41. H. Lehrach *et al.*, *Biochemistry* **16**, 4743 (1977).
42. We thank J. Gasson for helpful comments on the manuscript, S. G. Quan for artwork, C. Nishikubo, S. G. Quan, A. Healy, and J. Fujii for technical assistance, and H. Merriman, B. Koers and W. Aft for editorial assistance. Supported by NCI grants CA 30388, CA 32737, CA 38597, CA 09297, and CA 16042; by grants PF-2182 and JFRA-99 from the American Cancer Society, and by a Bank of America-Giannini Foundation Fellowship (W.W.).

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Predisposition to Hookworm Infection in Humans

Abstract. *Frequency distributions of parasitic helminths within human communities are invariably highly aggregated, the majority of worms occurring in relatively small fractions of the host populations. It has been suggested that the heavily infected individuals are predisposed to this state, not by chance, but by as yet undefined genetic, ecological, behavioral, or social factors. Analyses of individual post-treatment patterns of hookworm reinfection among 112 villagers in an endemic area of West Bengal provide quantitative evidence of predisposition to heavy infection. This observation has implications for the design of control programs based on chemotherapy because of the potential economic advantage of selective or targeted treatment as opposed to mass or blanket treatment.*

Epidemiological studies in various regions of the world have reported rates of post-treatment reinfection with hookworms and other intestinal helminths (*1*). Quantitative information on patterns of reinfection in individual patients, as opposed to groups of people, is extremely limited, however, and the evidence for predisposition to heavy infection is therefore inconclusive. This is in part a consequence of the small numbers of patients examined in previous studies (*2–5*). Here we present evidence of a statistically significant positive association between the adult worm burdens of individual patients prior to treatment (as judged by fecal egg counts) and the adult worm burdens in the same individuals following a period of reinfection.

Our analyses are based on field studies conducted in West Bengal, India, in 1968 to 1970 (*6–10*). The study area, its climate, methodological details (including methods of sample selection) and aspects of the epidemiology of hookworm parasitism are described elsewhere (*6–11*). Two groups of villagers from a rural area 40 miles (64 km) north of Calcutta were treated to remove their hookworms. Group 1 ($n = 34$) was treated with anthelmintics [bephenium hydroxynaphthoate and tetrachlorethylene (TCE)] in the dry season of 1968–1969 and then reexamined after 10, 12, 14, and 18 months. The monsoon season, a 4-month period during which conditions for frequent reinfection were optimal, began 6 months after treatment was completed. Group 2, the treated subsample of a study population of 735 villagers, was treated (with bephenium and either TCE or thiabendazole) in the same dry season and then reexamined at eight 2-month intervals (the total period included two monsoon seasons). People who provided fecal samples in at least four of

the eight post-treatment rounds of examination were included in our analyses (n = 78).

The frequency distributions of the two hookworm species present, *Ancylostoma duodenale* and *Necator americanus*, were aggregated as illustrated by the worm burden data from a combined sample of 84 people shown in Fig. 1, a and b (*12*). Observed patterns of worm numbers per person are well described empirically by the negative binomial probability distribution (*13*). Greater than 60 percent of the total hookworm population was harbored by less than 10 percent of the people in the combined sample.

The sharp post-treatment decrease in the mean intensity of infection to near zero (as judged by daily egg output; EPD), and the rapid return to about 30 percent of the pre-treatment level in 20 months is shown in Fig. 1c for group 2 (*14*). Statistically significant positive associations (at the 5 percent significance level) between individual pre- and post-treatment levels of infection were found in all rounds of reexamination (Table 1). The pre-treatment intensities in group 2, and the reinfection intensities at the final round of examination 630 days after treatment, are shown in Fig. 1d (*15, 16*).

Similar results were recorded for a smaller sample of 34 people in group 1. Reinfections occurred rapidly after treatment, and those who were heavily infected initially reacquired heavier than average infections after a 506-day period of reinfection (Kendall's coefficient of rank correlation, $\tau = 0.6694$, $P < 0.001$, n = 34). The similarity of the results from group 1 and group 2 is of interest given the between-group differences with respect to the timing of treatment, the chemotherapeutic agents used, and the period of time during which reinfection occurred. The results provide firm evidence of predisposition to heavy infection (or, conversely, light infection) (*2–5*).

Predisposition may result from ecological, genetic, social, or behavioral factors, either acting in isolation or in combination. For example, dense populations of infective larvae abound where human feces are habitually deposited on loamy soils in warm, moist, and well-shaded sites. Unshod individuals residing near such areas may tend to acquire heavy infections (*17*). Alternatively, social status or occupation may determine the degree of contact with fecally polluted soils. Similarly, behavioral factors, particularly those associated with personal hygiene and defecation habits, are likely to play a role in determining exposure to infection. Aggregated distributions of worm numbers per person and

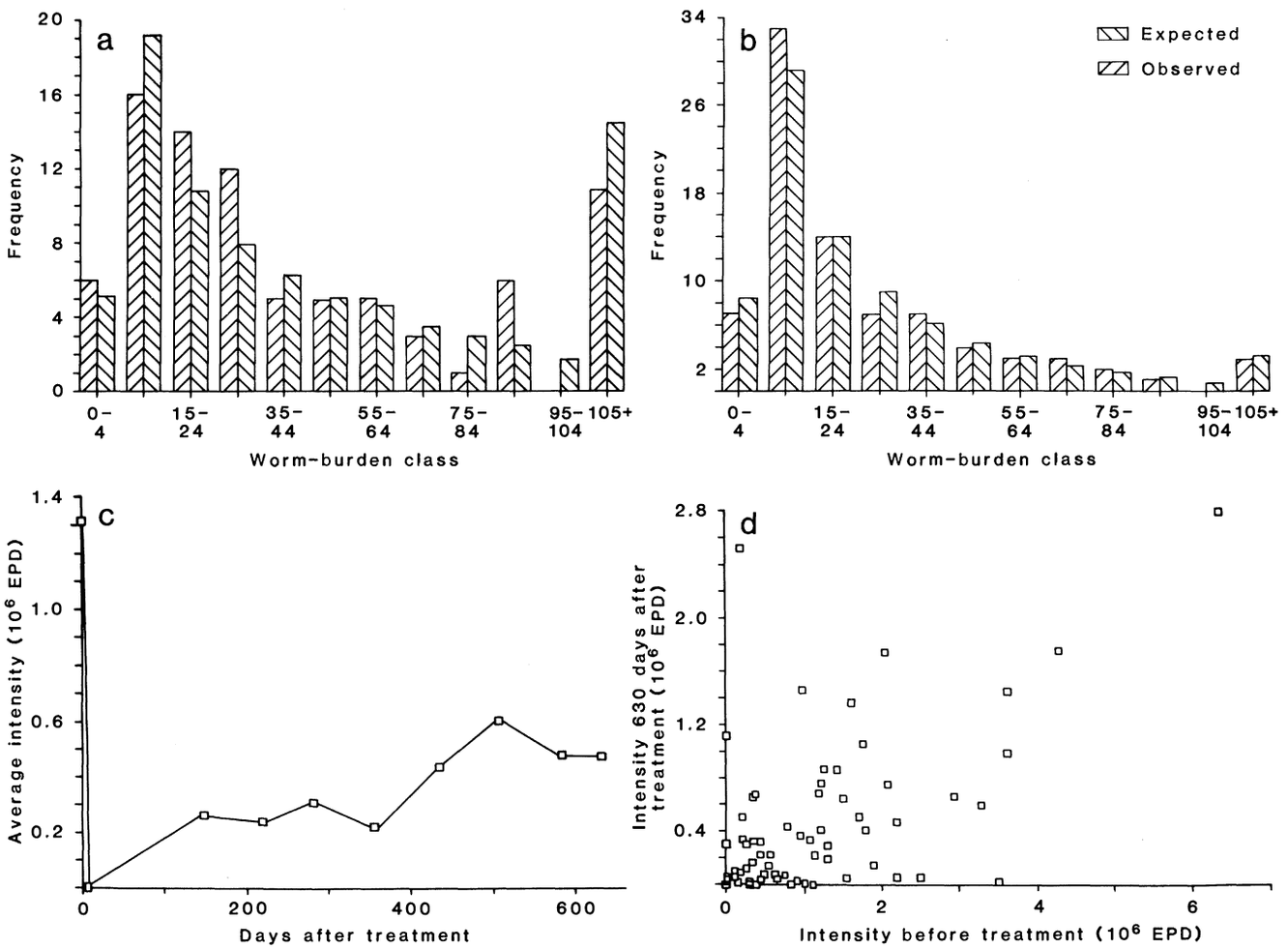

Fig. 1. (a) The frequency distributions of *A. duodenale* per person [groups 1 and 2 combined (*12*)]. Observed values are recorded as are the expected frequencies based on the fit of a negative binomial probability distribution to the observed data ($\bar{x}$ = 24.54, k = 0.618, n = 84, χ_{11}^2 = 4.24). (b) The frequency distributions (observed and expected) of *N. americanus* ($\bar{x}$ = 53.7, k = 0.627, n = 84, χ_{12}^2 = 12.0). (c) Infection in group 2 patients following multiple anthelmintic treatments (days 1 through 3) (*6–10*). The average intensity of infection is measured as the egg output per day (EPD) during eight rounds of examination at approximately 2-month intervals (*14*). Table 1 shows the number of patients examined and statistical comparisons. (d) The association between fecal egg counts (EPD) before treatment and those 630 days after treatment for a sample of group 2 patients (Kendall's rank correlation coefficient, τ = 0.33, n = 65, $P < 0.005$).

predisposition may therefore be explained on the basis of transmission-linked factors alone. Such explanations have gained wide acceptance in the absence of direct evidence for genetically controlled resistance mechanisms to hookworm infection (either immunological or nonspecific in nature) (*9, 18, 19*). However, with the demonstration of acquired resistance to hookworm infection in laboratory animals (*20*) and in some induced human infections (*19, 21*), variability in observed intensities of infection has been attributed to group and individual differences in resistance or susceptibility. Thus, for example, males appear to be inherently more susceptible than females (*8, 22–24*) and Caucasians more so than non-Caucasians (*25–27*). Laboratory models of human hookworm infection clearly suggest a genetic basis of resistance and susceptibility (*28*), but direct evidence of the significance of such factors for infection in humans is lacking. We suspect that both heterogeneity in exposure to infection within human communities and genetically determined host resistance mechanisms play important roles as determinants of parasite aggregation and host predisposition to infection.

Our analyses of individual patterns of reinfection do not provide information on the causal mechanisms of predisposition. Nevertheless, the significant positive association between pre- and post-treatment levels of infection has practical implications for the planning of hookworm control programs. Targeted anthelmintic treatment focusing on the ethnic and socioeconomic groups most heavily infected with hookworms has been effective in Fiji, the southern United States, and elsewhere (*26, 27, 29*). More narrowly focused community control by chemotherapy based on individuals, as opposed to groups, has not been attempted. Recently, the desirability of targeted or selective chemotherapy has been argued on both clinical and theoretical grounds (*4, 30, 31*). Mathematical models of the transmission dynamics of helminth infections suggest that selective treatment of heavily infected individuals can, in principle, provide cost-effective, community-wide hookworm control (*4*). However, weighed against the superficial cost advantages of selective treatment (fewer drug treatments required when compared with mass or blanket application within a community) are the additional expenses arising from the identification of heavily infected individuals. To a large extent, such considerations have prevented the wide-scale acceptance of selective treatment as a practical approach to community control of helminth infections (*31*). Our evidence for predisposition to heavy hookworm infection, however, argues for a reappraisal of these issues. Once identified, treatment could be continually focused on those predisposed to heavy infection, thereby eliminating the necessity of repeated identification at each round of drug administration (*4, 32*).

Table 1. Kendall's rank correlation coefficient (τ) for patients in group 2 showing the degree of association between individual worm burdens (as measured by fecal egg counts) before treatment and at eight 2-month intervals after treatment. The value of n is the number of people examined at each round; all values of τ were significant at the 1 percent level employing a Bonferroni correction.

	n	τ
Pretreatment	78	1.00000
Round 1	63	0.39416
Round 2	67	0.38617
Round 3	66	0.38425
Round 4	73	0.25593
Round 5	67	0.37853
Round 6	70	0.37042
Round 7	63	0.39414
Round 8	65	0.32924

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References and Notes

1. W. W. Cort, L. Schapiro, N. R. Stoll, *Am. J. Hyg.* **10**, 614 (1929); J. F. Docherty, *ibid.* **6**, 160 (1926); M. Bhaibulaya *et al.*, *Southeast Asian J. Trop. Med. Public Health* **8**, 329 (1977); R. B. Hill, *South. Med. J.* **18**, 665 (1925); S. M. Lambert, *J. Trop. Med. Hyg.* **31**, 113 (1928); *ibid.* **39**, 19 (1936); W. C. Sweet, *Am. J. Hyg.* **4**, 691 (1924); *Ceylon J. Sci.* (section D, Medical Science) **1**, 129 (1925); F. Arfaa and E. Ghadirian, *Am. J. Trop. Med. Hyg.* **26**, 866 (1977); N. A. Croll, R. M. Anderson, T. W. Gyorkos, E. Ghadirian, *Trans. R. Soc. Trop. Med. Hyg.* **76**, 187 (1982); R. B. Hill, *Am. J. Hyg.* (Suppl. *2*), 103 (1926).
2. H. M. Gilles, E. J. Watson Williams, P. A. J. Ball, *Q. J. Med.* **33**, 1 (1964).
3. N. A. Croll and E. Ghadirian, *Trop. Geogr. Med.* **33**, 241 (1981).
4. R. M. Anderson and R. M. May, *Nature (London)* **297**, 557 (1982).
5. Predisposition to heavy infection has been demonstrated for helminth parasites (*Nematodirus spathiger*) in sheep. See J. K. Dineen, A. D. Donald, B. M. Wagland and J. H. Turner, *Parasitology* **55**, 103 (1965).
6. G. A. Schad *et al.*, *Science* **180**, 502 (1973).
7. G. A. Schad, E. J. L. Soulsby, A. B. Chowdhury, H. M. Gilles, in *Nuclear Techniques in Helminthology Research* (International Atomic Energy Agency, Vienna, 1975), pp. 41–54.
8. T. A. Nawalinski, G. A. Schad, A. B. Chowdhury, *Am. J. Trop. Med. Hyg.* **27**, 1152 (1978).
9. G. A. Schad, T. A. Nawalinski, V. K. Kochar, in *Human Ecology and Infectious Disease*, N. A. Croll and J. Cross, Eds. (Academic Press, New York, 1984), pp. 188–223.
10. For methodological details, see (*6–9*); for sample selection and details of anthelmintic treatment, see R. M. Anderson and G. A. Schad, *Trans. R. Soc. Trop. Med. Hyg.*, in press.
11. A. B. Chowdhury and E. L. Schiller, *Am. J. Epidemiol.* **87**, 299 (1968).
12. The frequency distributions (Fig. 1, a and b) are based on counts of hookworms expelled after treatment. Because few people would save their full fecal output for 72 hours after treatment, worm-count data included patients from groups 1 and 2, as well as others from the same study population of 735 villagers from which group 2 was selected, who were treated later in the investigation. Thus, the 84 people forming the combined sample were all those providing complete worm-count data. For raw data, see appendix in Anderson and Schad (*10*).
13. See R. M. Anderson, *Population Dynamics of Infectious Diseases: Theory and Applications* (Chapman & Hall, London, 1982), for further details of frequency distributions of helminth parasites in host populations.
14. Combined treatment with bephenium hydroxynaphthoate and either tetrachlorethylene or thiabendazole (*6–10*) removed all worms from 50 percent of the patients who provided fecal samples for post-treatment examination (n = 62). The heavily infected half of the group (n = 31) did not differ from the lightly infected half in the number of individuals becoming negative by fecal examination (χ^2 = 0.58, d.f. = 1). Even when only the most heavily and the most lightly infected 25 percent were compared (n = 15 for each), the two groups did not differ with respect to the number of individuals becoming negative (χ^2 = 0.53, d.f. = 1). The post-treatment fecal examinations (as distinct from those done in the regularly scheduled rounds) were planned to identify patients completely cleared of adult hookworms and, therefore, particularly sensitive qualitative (rather than quantitative) techniques were used to preclude the occurrence of false negative examinations (*6–10*). The EPD data were available for 27 individuals. When these individuals were grouped by pretreatment EPD into the most heavily infected (n = 14) and the most lightly infected (n = 13) subgroups, no difference in the residual, post-treatment EPD was demonstrated (t = 0.87, d.f. = 25). These analyses indicate that the positive associations between pre- and post-treatment levels of infection (Table 1 and Fig. 1d) do not merely reflect higher post-treatment residual egg outputs in the more heavily infected than the more lightly infected individuals.
15. The increasing post-treatment hookworm egg counts (Fig. 1c) reflect reinfection with adult *A. duodenale* and, particularly, with *N. americanus*, the dominant species in our study area (*11*). In the case of *N. americanus*, rising egg counts represent simple reinfection from the external environment because its infective larvae do not persist in the host's extraintestinal tissues. In the case of *A. duodenale*, however, the increasing counts reflect both simple reinfection from the exterior, as well as intestinal reinfection due to the reactivation of persistent arrested larvae surviving from before treatment. These surviving larvae probably contributed substantially to the initial post-treatment increase in egg count (Fig. 1c) because they would have emerged from arrest in the premonsoon period (*6, 16*). This period was included between treatment and the first post-treatment round of fecal examination. It is unlikely, however, that larvae becoming arrested in the pretreatment period contributed substantially to the overall increase in egg output because: (i) most of the resulting adult worms would have been expelled within the first 12 of the 21 months represented (*6, 16*), and (ii) these adults would have been replaced from the new populations of larvae entering the host population during the two post-treatment monsoon seasons included in the overall timespan of our investigation. Thus, most reinfection depicted in Fig. 1c is, in fact, "true" reinfection from the external environment.

    Figure 1d compares pretreatment egg counts with final counts at 630 days after treatment. Because arrested *A. duodenale* larvae resume development seasonally and the adult population turns over annually (*9*), pretreatment arrested larvae would have influenced these data minimally, if at all. Indeed, from the standpoint of control of either morbidity or transmission, predisposition to adult worm parasitism is of primary importance; whether these worms are derived from an internal or external source of infective (L3) larvae is largely a matter of academic interest.
16. T. A. Nawalinski and G. A. Schad, *Am. J. Trop. Med. Hyg.* **23**, 895 (1974); _____, A. B. Chowdhury, *ibid.* **27**, 1162 (1978).
17. W. W. Cort, N. R. Stoll, W. C. Sweet, W. A.

Riley, L. Schapiro, *Am. J. Hyg. Monogr. Ser.* **9** (1929).
18. A. C. Chandler, *Hookworm Disease* (Macmillan, New York, 1929), 429 pp.
19. T. A. Miller, *Adv. Parasitol.* **17**, 315 (1979).
20. ———, *J. Parasitol.* **52**, 512 (1962); *Immunology* **12**, 231 (1967); *Adv. Parasitol.* **9**, 153 (1971).
21. W. W. Cort and G. F. Otto, *Rev. Gastroenterol.* **7**, 2 (1940).
22. L. K. Martin, *Am. J. Trop. Med. Hyg.* **21**, 930 (1972).
23. J. G. Banwell and G. A. Schad, *Clin. Gastroenterol.* **7**, 129 (1978).
24. G. A. Schad and J. G. Banwell, in *Tropical and Geographic Medicine*, A. A. F. Mahmoud and K. S. Warren, Eds. (McGraw-Hill, New York, 1984), pp. 359–372.
25. H. H. Howard, *South. Med. J.* **18**, 668 (1925).
26. J. M. Andrews, *Am. J. Public Health* **32**, 282 (1942).
27. J. M. Andrews, *Ann. Intern. Med.* **17**, 891 (1942).
28. A. Bartlett and P. A. J. Ball, *Ann. Trop. Med. Parasitol.* **66**, 129 (1972); D. Wakelin, *Adv. Parasitol.* **16**, 219 (1978).
29. S. M. Lambert, *J. Trop. Med. Hyg.* **39**, 19 (1936).
30. K. S. Warren and A. A. F. Mahmoud, *Clin. Res.* **28**, 195 (1970).
31. K. S. Warren, *Annu. Rev. Public Health* **2**, 101 (1981).
32. R. M. Anderson and R. M. May, *Adv. Parasitol.*, in press.
33. Fieldwork in India was supported by foreign research agreement 01-027-01 and grant 5 RO 7 TW00141 from the U.S. Public Health Service. Data analysis was supported by a fellowship from the Welcome Trust. We thank G. Medley for assistance with data processing, C. Kirkpatrick for critical reading of the manuscript, and S. Shaw, S. Pharaoh and L. Hodges for assistance with the preparation of the manuscript.

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Analysis of T-Cell Receptor Gene Rearrangement and Expression in Human Natural Killer Clones

Abstract. *A series of clones of human natural killer (NK) cells was characterized with respect to expression of the Ti α and Ti β genes of the T-cell receptor. $T11^+T3^+$ NK clones contained Ti α and Ti β RNA transcripts and expressed disulfide-linked heterodimers, demonstrating the presence of a functional T-cell receptor. In contrast, $T11^+T3^-$ NK clones expressed only 1.0-kilobase truncated Ti β transcripts, without a Ti α transcript and no detectable surface Ti protein. Since previous studies demonstrated that Ti β gene activation precedes Ti α gene activation in thymic ontogeny, the $T11^+T3^-$ NK cells appear to be derived from T-lineage precursors.*

Natural killer (NK) cells have been operationally defined as a population of cells capable of mediating direct cytotoxicity against various types of target cells without apparent prior immunization (*1*). Primarily because the cytotoxic specificity of NK cells is not restricted by or associated with expression of major histocompatibility complex (MHC) antigens on their target cells, NK cells have been thought to be functionally distinct from cytotoxic T lymphocytes. In peripheral blood, NK cells appear to be a morphologically homogeneous population of large granular lymphocytes (LGL) that can be distinguished and separated from conventional T cells by their differing physical characteristics (*2*). Nevertheless, the lineage of NK cells has not been established since they express both T lymphocyte– and myeloid-associated cell surface antigens. Moreover, the structural basis by which these cells exert their cytotoxic function has not been defined for most of the NK active cells.

In order to analyze NK cell function and heterogeneity, we and others have developed and characterized monoclonal NK cell lines from normal peripheral blood (*3*). This is possible because NK cells proliferate in response to nonspecific stimulation, and this proliferation can be maintained by interleukin-2. Using these techniques, we were able to select a series of NK clones solely on the basis of their ability to mediate cytotoxicity against various types of target cells without prior sensitization. Previous characterization of these NK clones has shown that they can maintain a stable phenotype and cytotoxic function after prolonged periods of culture in vitro. Moreover, these NK clones appear to reflect accurately the phenotypic and functional diversity of NK cells in unstimulated peripheral blood. In the present experiments, we used DNA probes for the Ti α (*4*) and Ti β (*5*) genes of the T-cell receptor to analyze various NK clones for the presence of specific T-cell lineage rearrangements as well as expression of T-cell receptor gene products. These studies were undertaken to further characterize the derivation of NK cells and to analyze the structural basis for the cytotoxic function of these cells.

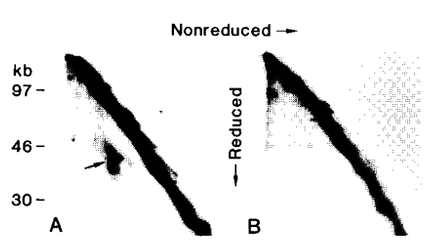

Fig. 1. Two-dimensional SDS-PAGE of NK clones labeled at surface membranes with ^{125}I. (A) JT9 cells. (B) JT3 cells. Lymphoid cells were externally labeled with ^{125}I-labeled lactoperoxidase, and total cell lysates from 5×10^6 to 10×10^6 cells were analyzed by modification of the procedure described by Goding and Harris (*10, 11*). Lysates were first resolved under nonreducing conditions in a 10 percent acrylamide Laemmli tube gel. When the bromophenol blue marker reached the bottom of the tube, the gel was removed and equilibrated for 1 hour at 25°C in sample buffer containing 10 percent 2-mercaptoethanol and 100 m*M* dithiothreitol. Samples were then subjected to electrophoresis in the second dimension under reducing conditions in a 10 percent acrylamide Laemmli slab gel. Gels were fixed and autoradiographed with intensifying screens and Kodak X-R5 film at −70°C for 48 hours. The arrow in (A) indicates the position of the disulfide-linked heterodimer. A similar spot is not detectable in (B).

The phenotype of the NK clones used in our studies is summarized in Table 1. Clones JT3 and JT_B18 represent the phenotype of most of the NK cells in peripheral blood (*6*). These cells express T11/E rosette antigen and NKH1, a pan-NK cell antigen, but do not express T3, T4, or T8 antigens. Although these two clones have a similar phenotype, they were derived from different individuals, and only JT3 cells express NKH2 antigen—another marker associated with LGL in peripheral blood (*6*). Clones JT3, JT9, and JT10 were derived at separate times from the same individual, but JT9 and JT10, in contrast to JT3, have a mature T-cell phenotype ($T3^+$, $T8^+$, $T11^+$). Unlike most T lymphocytes, however, these cells express NKH1 antigen and kill a wide variety of target cells including K562. Cytotoxicity of JT9 and JT10 cells is not MHC-restricted and cannot be blocked by monoclonal antibodies specific for T8 structures or for histocompatibility antigen class I or class II products (*7*). Clones JT9 and JT10 demonstrate an identical target specificity to each other, and both express a 90-kilodalton (kD) clonotypic disulfide-linked heterodimeric receptor (NKTa) that is linked to surface T3 antigen and appears to be the product of functional T-cell receptor genes (*8*). Cytotoxicity of JT9 and JT10 cells can be blocked at the effector cell level by monoclonal antibodies specific for NKTa or T3 antigens and at the target cell level by monoclonal antibodies specific to TNK_{TAR}, a 140-kD glycoprotein that is widely expressed on normal lymphoid and hematopoietic cells after cell activation (*9*). Clone CNK3 was derived from a different individual and also has a mature T-cell phenotype ($T3^+$,$T8^+$, and $T11^+$). Furthermore, its cytotoxicity is also blocked by antibody to T3. Unlike JT9 and JT10, these cells express NKH2 antigen and do not express NKTa.

The possibility that NK clones lacking T3 express a disulfide-linked heterodi-