thase. These findings support the concept that insulin mediators are involved in the rapid intracellular responses to insulin but probably are not involved in membrane transport processes. Consistent with this concept is the finding of Gelehrter *et al.* (*29*) that the mechanism responsible for insulin stimulation of ion transport in rat hepatoma cells differs from the postbinding mechanisms involved in the stimulation of amino acid transport and tyrosine aminotransferase induction.

Two recent reports would indicate that insulin receptor phosphorylation is not involved in the mechanism responsible for insulin stimulation of glucose transport. Plehwe *et al.* (*30*) showed that marked suppression of insulin-stimulated phosphorylation had little if any effect on insulin-stimulated glucose transport. Simpson and Hedo (*31*) reported that an antiserum to the insulin receptor mimicked insulin's action on glucose transport, phosphorylation of integral membrane proteins, and internalization of the insulin receptor in fat cells, while having no effect on phosphorylation of the subunit of the insulin receptor. Thus, yet to be defined mechanisms must be involved in insulin stimulation of glucose transport. Experiments involving subcellular systems, intact cells, and whole animals will be needed to define the exact role of the insulin mediators in each of insulin's actions. At the same time, major efforts are needed to purify and chemically identify this apparently new family of mediator substances.

LEONARD JARETT
Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia 19104

ELLEN H. A. WONG
Department of Biochemistry, National University of Singapore, Kent Ridge, Singapore 0511

S. LANCE MACAULAY
JUDITH A. SMITH
Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine

References and Notes

1. L. Jarett, F. L. Kiechle, J. C. Parker, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 273 (1982); L. Jarett, F. L. Kiechle, J. C. Parker, S. L. Macaulay, *Am. J. Med.* **74**, 31 (1983); J. Larner, *J. Cyclic Nucl. Res.* **8**, 289 (1982); J. R. Seals and M. P. Czech, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 2730 (1982).
2. J. Larner, *Am. J. Med.* **74**, 38 (1983).
3. J. R. Seals, J. M. McDonald, L. Jarett, *J. Biol. Chem.* **254**, 6991 (1979); *ibid.*, p. 6997.
4. L. Jarett and J. R. Seals, *Science* **206**, 1407 (1979).
5. J. R. Seals and L. Jarett, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 77 (1980).
6. J. R. Seals and M. P. Czech, *J. Biol. Chem.* **255**, 6529 (1980).
7. F. L. Kiechle, L. Jarett, D. A. Popp, N. Kotagal, *ibid.* **256**, 2945 (1981).
8. A. Saltiel, S. Jacobs, M. Siegel, P. Cuatrecasas, *Biochem. Biophys. Res. Commun.* **102**, 1041 (1981).
9. Y. Sakamoto, T. Kuzuya, J. Sato, *Biomed. Res.* **3**, 599 (1982).
10. N. Begum, H. M. Tepperman, J. Tepperman, *Endocrinology* **110**, 1914 (1982).
11. J. M. Amatruda and C. L. Chang, *Biochem. Biophys. Res. Commun.* **112**, 35 (1983).
12. F. L. Kiechle, L. Jarett, D. Popp, N. Kotagal, *Diabetes* **29**, 852 (1980).
13. J. C. Parker, F. L. Kiechle, L. Jarett, *Arch. Biochem. Biophys.* **215**, 339 (1982).
14. L. Jarett, F. L. Kiechle, D. A. Popp, N. Kotagal, J. R. Gavin III, *Biochem. Biophys. Res. Commun.* **96**, 735 (1980).
15. J. Larner *et al.*, *Science* **206**, 1408 (1979).
16. J. C. Parker and L. Jarett, *Diabetes*, in press.
17. J. R. Seals and M. P. Czech, *ibid.*, p. 2894; D. Popp, F. L. Kiechle, N. Kotagal, L. Jarett, *J. Biol. Chem.* **255**, 7540 (1980).
18. J. Larner *et al.*, *Recent Prog. Horm. Res.* **38**, 511 (1982).
19. F. L. Kiechle and L. Jarett, *FEBS Lett.* **133**, 279 (1981).
20. A. R. Saltiel, A. Doble, S. Jacobs, P. Cuatrecasas, *Biochem. Biophys. Res. Commun.* **110**, 789 (1983).
21. A. R. Saltiel, M. I. Siegel, S. Jacobs, P. Cuatrecasas, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3513 (1982).
22. S.-R. Zhang, Q.-H. Shi, R. J. Ho, *J. Biol. Chem.* **258**, 6471 (1983).
23. J. F. Caro, F. Folli, F. Cecchin, M. K. Sinha, *Biochim. Biophys. Res. Commun.* **115**, 375 (1983).
24. L. Jarett, E. H. A. Wong, J. A. Smith, S. L. Macauley, *Endocrinology*, in press.
25. M. Rodbell, *J. Biol. Chem.* **239**, 375 (1964).
26. L. Mukherjee and R. L. Jungas, *Biochem. J.* **148**, 229 (1975); R. M. Denton and W. A. Hughes, *Int. J. Biochem.* **9**, 545 (1978); J. M. May and C. de Haan, *J. Biol. Chem.* **254**, 9017 (1979); S. P. Mukherjee and C. Mukherjee, *Arch. Biochem. Biophys.* **214**, 211 (1982).
27. J. C. Lawrence, Jr., and J. Larner, *Mol. Pharmacol.* **14**, 1079 (1978).
28. C. Ludvigsen, L. Jarett, J. M. McDonald, *Endocrinology* **106**, 786 (1980).
29. T. D. Gelehrter *et al.*, *Diabetes* **33**, 428 (1984).
30. W. E. Plehwe *et al.*, *Biochem. J.* **214**, 361 (1983).
31. I. A. Simpson and J. A. Hedo, *Science* **223**, 1301 (1984).
32. F. L. Kiechle and L. Jarett, *Mol. Cell. Biochem.* **56**, 99 (1983).
33. J. A. Thomas, K. K. Schlender, J. Larner, *Anal. Biochem.* **25**, 486 (1968); *Biochim. Biophys. Acta* **193**, 84 (1973).
34. L. Jarett and R. M. Smith, *J. Biol. Chem.* **249**, 7024 (1974).
35. Supported in part by NIH grants AM28144 and AM19525 and a grant from the Reynolds Foundation. S.L.M. is supported by a grant from the Juvenile Diabetes Foundation and E.H.A.W. is a fellow of the American Association of University Women. We acknowledge the technical contribution of J. O. Macaulay.

16 July 1984; accepted 7 December 1984

Vaccination Against *Schistosoma mansoni* with Purified Surface Antigens

Abstract. *Two surface antigens were isolated from young or adult schistosomes by affinity chromatography with monoclonal antibodies. Vaccination with an antigen having a molecular weight of 155,000 gave partial protection against challenge in some batches of mice and in a group of cynomolgus monkeys. Vaccination with an antigen having a molecular weight of 53,000 gave similar levels of protection in mice. The results demonstrate that protection can be obtained with single antigens, but the precise requirements for reproducible vaccination are as yet unknown.*

Schistosomiasis is a debilitating tropical parasitic disease that affects more than 200 million people in 73 countries. The causal agents are small paired worms that live in the hepatic portal system and produce large numbers of eggs, which escape via the intestinal wall and feces and hatch in fresh water. The miracidial stage that emerges from the egg infects certain species of snails and multiplies asexually in this intermediate host. Man is infected by direct penetration of the skin by the cercarial stage, which emerges from the snail into water.

An infection can induce substantial, though not complete, resistance against reinfection in several laboratory hosts, and there is circumstantial evidence that immunity develops in man (*1*, *2*). Vaccination with large numbers of living juvenile schistosomes, irradiated to restrict development, can stimulate high levels of protection (*3*), but most attempts to vaccinate with extracted material have been only marginally effective (*4*). One unconfirmed report indicates that extracts of cercariae, the infective stage, can give significant protection in mice (*5*). In vitro studies and experiments with irradiated cercariae have shown that the target antigens are located on the surface membrane of the young schistosomula stage, which is formed as soon as the cercariae penetrate the skin (*2*). There is considerable interest in characterizing individual surface antigens with monoclonal antibodies (*6*), and several groups are moving toward the production of target antigens by genetic engineering in order to obtain sufficient quantities for vaccination studies (*7*). Although large-scale production of antigens will almost certainly require biosynthesis or synthesis of short peptides, quantities sufficient for experimental vaccination can be obtained from moderate numbers of schistosomulae or adult schistosomes. We report here the isolation of two surface antigens by affinity chromatography with monoclonal antibodies and present preliminary results of vaccination trials.

Eight mouse monoclonal antibodies directed against surface antigens of schistosomulae were available, but only one of these, an immunoglobulin M (IgM) antibody (WP66.4), mediates a sig-

nificant level of protection against challenge in passive transfer experiments (*8*). This IgM monoclonal antibody was shown in Western blot experiments to recognize an antigen with a molecular weight of about 155,000 in extracts of adult parasites (Fig. 1). Western blots of schistosomula proteins did not reveal any antigens detected by this antibody, but affinity chromatography allowed isolation of very small quantities of a 155K antigen from detergent extracts of schistosomulae.

Initial attempts to prepare an effective immunoabsorbent with monoclonal antibody 66.4 were discouraging until it was realized that this IgM has a pronounced tendency to aggregate at *p*H 8.0 during purification but not at *p*H 7.0. Partial purification of the IgM by gel filtration at *p*H 7.0 and coupling to activated Sepharose 4B at the same *p*H gave a very efficient immunoabsorbent that could be used repeatedly for isolation of pure 155K antigen from detergent extracts of adult schistosomes (see legend to Fig. 2). The yield of antigen was approximately 60 μg from a batch of 250 adult parasites. A much lower yield of the antigen was obtained from a batch of 100,000 schistosomulae. A schistosomula surface antigen of about the same molecular weight (160K) has been detected (*9*) with an immunoglobulin G2b (IgG2b) mouse monoclonal antibody raised against parasite eggs.

A second surface antigen was isolated from detergent extracts of schistosomulae by affinity chromatography on immunoabsorbent prepared from an immunoglobulin G3 (IgG3) monoclonal antibody (WP80.6). The apparent molecular weight of the antigen was 53K, with a yield of 70 μg from 100,000 schistosomulae (Fig. 3). An antigen of the same molecular weight could be obtained from extracts of adult schistosomes, but it was accompanied by two other proteins that could not be removed from the immunoabsorbent by a variety of washing procedures. Monoclonal antibody 80.6 did not bind to any antigen in Western blots of adult or schistosomula extracts, and it is not clear whether these additional proteins are genuinely recognized by the antibody or represent strongly binding nonspecific proteins. Because of problems encountered during isolation of the 53K antigen from adult schistosomes, this antigen was routinely purified from schistosomulae for experimental vaccination in spite of the difficulty in preparing large numbers of this stage.

Although both antigens can be detected on the surface membrane of living schistosomulae by the appropriate monoclonals (*8*) or by polyclonal antibodies raised in mice by immunization with the purified antigens, they apparently are not synthesized by this early stage of the parasite. Isolation of the 155K and 53K antigens from detergent extracts of schistosomulae that had been cultured for 16 hours in medium containing [^{35}S]methionine, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography (*10*), showed that neither antigen had been labeled. Both antigens incorporated the label, however, when adult schistosomes were cultured under the same conditions. Experiments involving cell-free translation of messenger RNA from various stages of *Schistosoma mansoni* have also shown that two other

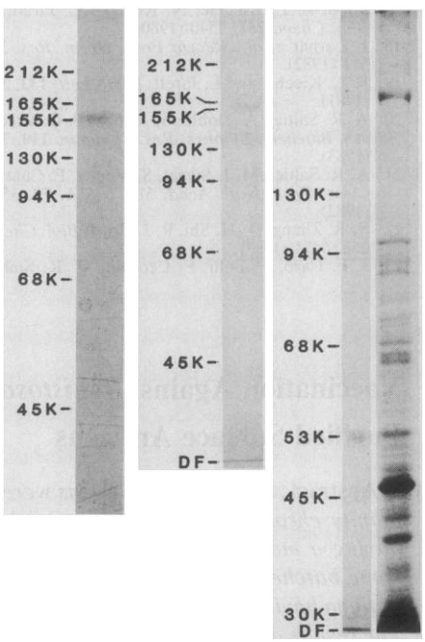

Fig. 1 (left). Identification of the antigen recognized by IgM monoclonal antibody 66.4 by Western blot. Adult schistosomes (50 pairs) were extracted by boiling for 10 minutes in 100 μl of 62 m*M* tris-HCl buffer (*p*H 6.8) containing 2 percent SDS, 10 percent (by volume) glycerol, 2 m*M* phenylmethylsulfonyl fluoride, 0.25 *M* dithiothreitol, and 0.05 percent bromophenol blue. Polypeptides were separated on 7.5 percent polyacrylamide gels containing 0.1 percent SDS with a 3.5 percent stacking gel in the discontinuous system of Laemmli (*16*) and transferred electrophoretically to nitrocellulose paper (*17*). Nitrocellulose strips were reacted with monoclonal antibody 66.4 (1:20, 1.9 mg of IgM per milliliter) and washed with 0.1*M* tris-HCl (*p*H 7.0) containing 0.1 percent Tween 20. Bound antibody was detected with ^{125}I-labeled F(ab')$_2$ fragment of rabbit antibody to mouse IgG, by autoradiography (Kodak X-Omat x-ray film). Molecular weight marker polypeptides were myosin (212K), *Escherichia coli* RNA polymerase (165K, β^1 subunit and 155K β subunit), β-D-galactosidase (130K), phosphorylase (94K), bovine serum albumin (68K), and ovalbumin (45K). Fig. 2 (center). Preparative purification of the 155K antigen by affinity chromatography with monoclonal antibody 66.4. The antibody was partially purified from mouse ascitic fluid (2 to 4 mg of IgM per milliliter) by gel filtration on Sephacryl S-300 (Pharmacia) by using 0.1*M* sodium phosphate buffer (*p*H 7.0) containing 0.5*M* NaCl. Fractions rich in IgM were dialyzed against 0.05*M* sodium bicarbonate containing 0.15*M* NaCl, brought to *p*H 7.0 by gassing with CO_2, and coupled to CNBr-activated Sepharose 4B in a sealed container to maintain the correct *p*H (1.9 mg of IgM per milliliter of gel). Further treatment of the immunoabsorbent followed the recommendations of the manufacturer (Pharmacia). Batches of 250 adult worms were gently homogenized and extracted for 1 hour at 4°C in 1 ml of 10 m*M* tris-HCl (*p*H 7.0) containing 0.5 percent Nonidet P$_{40}$ (NP$_{40}$), 5 m*M* iodoacetamide, 2 m*M* phenylmethylsulfonyl fluoride, 1 m*M* EDTA, and 1 m*M* EGTA. The extract was centrifuged at 130,000*g* for 10 minutes in a Beckman Airfuge and applied to a 10-ml column of immunoabsorbent equilibrated with 10 m*M* tris-HCl (*p*H 7.0) containing 0.1 percent NP$_{40}$ and 1 m*M* EDTA. The immunoabsorbent was washed successively with (i) ten column volumes of the same buffer containing 0.3*M* NaCl and 1 percent NP$_{40}$ and (ii) two volumes of sodium acetate buffer (*p*H 4.0) and was then returned to the equilibrium buffer. Specifically bound antigen was eluted with 2*M* KSCN in the same buffer. After dialysis against equilibration buffer the eluate was reapplied to the column and retained antigen was eluted as before, dialyzed, and lyophilized. Antigen was dissolved in 62 m*M* tris-HCl (*p*H 6.8) containing 8*M* urea and 2 percent SDS by heating at 100°C for 10 minutes, and purity was assessed by SDS-PAGE on 7.5 percent gels (*16*). Polypeptides were fixed and stained in Coomassie blue R-250. The figure shows the single antigen isolated with monoclonal antibody 66.4. Molecular weight marker polypeptides are as in Fig. 1 (DF, dye front). Fig. 3 (right). Preparative purification from schistosomulae of the antigen recognized by monoclonal antibody 80.6. Monoclonal 80.6 was purified from ascitic fluid (5 mg of IgG3 per milliliter) by affinity chromatography with Protein A (*18*) and coupled to CNBr-activated Sepharose 4B at 3 mg of IgG3 per milliliter of gel by the method of the manufacturer, Pharmacia. Batches of about 100,000 schistosomulae prepared by a mechanical method (*19*) were cultured in vitro overnight and then gently homogenized and solubilized in extraction buffer (described in the legend to Fig. 2) at *p*H 8.0 and applied to the immunoabsorbent equilibrated in 10 m*M* tris-HCl (*p*H 8.0) containing 0.1 percent NP$_{40}$ and 1 m*M* EDTA. The column was then washed with ten volumes of the same buffer containing 0.5*M* NaCl and 1 percent NP$_{40}$ and returned to the equilibration buffer. Specific antigen was eluted with 50m*M* diethylamine at *p*H 11.5, neutralized with glycine, dialyzed, rechromatographed, and finally dialyzed and lyophilized. Antigen was tested for purity by SDS-PAGE on a 7.5 percent gel. Standard polypeptides were phosphorylase 94K; bovine serum albumin, 68K; glutamate dehydrogenase, 53K; ovalbumin, 45K; and carbonic anhydrase, 30K. Lane 1: 53K antigen recognized by monoclonal 80.6; lane 2, total schistosomula extract.

schistosomula surface antigens with molecular weights of 22K and 14K are not synthesized by young schistosomulae (*7*).

The results of vaccinating mice with the 155K or 53K schistosome surface antigens are summarized in Table 1. Two doses of antigen in alhydrogel adjuvant were given at an interval of 4 weeks, and 2 weeks later the mice were challenged by infection with cercariae. The effectiveness of the vaccine was estimated by comparing the number of adult worms recovered from groups of vaccinated mice 6 weeks after infection with the number recovered from control groups "vaccinated" with the adjuvant alone. Comparison of the mean number of parasites recovered from all control groups (34.4 ± 1.3) with the mean for all vaccinated groups (25.7 ± 1.1) showed that vaccination with the purified antigens had a significantly protective effect ($P < 0.01$) (Fig. 4). The wide random variation between different groups of vaccinated mice obscures any effect that might have been expected from the amount of antigen in the vaccine. Vaccinating three cynomolgus monkeys twice with 200 μg of the 155K antigen in alhydrogel gave a significant degree of protection against a cercarial challenge relative to two control monkeys injected with the adjuvant alone (*11*).

These results demonstrate that partial protection against a challenge infection can be obtained by vaccination with purified surface antigens isolated from adult worms or schistosomulae. Although this finding is a step toward an effective vaccine for use in man, the relatively low levels of protection obtained and the lack of consistency that we encountered are serious obstacles.

Vaccination with large numbers of irradiated cercariae, which die at a relatively early stage of development, can give high levels of protection in mice (*3*), and the reproducibility of these results is very much higher than that obtained with purified antigen. The mechanism of resistance induced by infection with irradiated organisms is imperfectly understood, but there is considerable evidence for involvement of antibodies against schistosomulae and a cellular component—possibly activated macrophages—with a nonspecific effector mechanism (*12*).

We have found that vaccination with purified surface antigens of schistosomulae stimulates a strong antibody response, but we have not examined vaccinated mice for activated macrophages capable of killing schistosomulae in vitro. Failure to stimulate this cellular arm of the response may be responsible for the relatively low and variable levels of protection induced by vaccination with purified antigens. Alternatively, the important variable may involve the class of antibody stimulated by vaccination. It has been suggested (*5*) that immunoglobulin E (IgE) is an essential feature of successful vaccination against schistosomes, but experiments with mice defective in IgE responses (*13*) have excluded the participation of antibodies of this class in resistance induced by vaccination with irradiated cercariae. In our experiments, groups of vaccinated mice had raised levels of all subclasses of IgG and IgM antibodies but very little increase in IgE (*14*).

Progress toward an effective nonliving vaccine against schistosomiasis may depend as much on the discovery of the correct immune response that must be stimulated as on production of a suitable antigen by genetic engineering techniques or direct peptide synthesis.

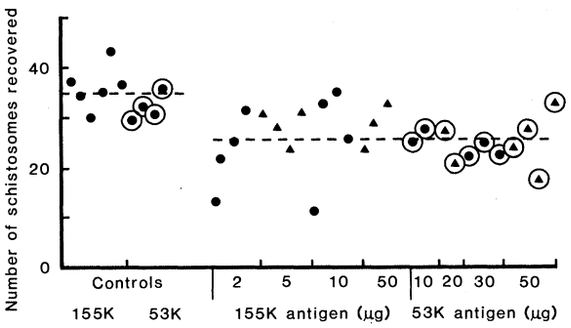

Fig. 4. Comparison of the mean number of schistosomes recovered from all control and vaccinated groups of mice (Table 1). The dotted lines indicate the control mean ± standard error (34.4 ± 1.3) and the mean for all vaccinated groups (27.1 ± 1.1). The means are significantly different at $P < 0.01$ (Student's *t*-test).

Table 1. Vaccination of B6D2 F_1 hybrid mice with schistosomula surface antigens with molecular weights of 155K or 53K. Groups of ten mice received two intramuscular injections of antigen in alhydrogel adjuvant (Superfos a/s), with an interval of 4 weeks elapsing between injections. Two weeks later vaccinated mice and control groups "vaccinated" with alhydrogel alone were challenged with approximately 100 cercariae, and 6 weeks later adult schistosomes were recovered from the portal system by perfusion (*15*).

Experiment	Dose of antigen (μg)	Number of schistosomes recovered (mean ± standard error)
	155K antigen	
1	2	13.1 ± 1.3
	5	30.8 ± 2.4
	0	37.2 ± 1.5
2	2	22.2 ± 1.9
	5	28.1 ± 1.3
	0	34.4 ± 1.3
3	2	25.1 ± 1.1
	10	11.2 ± 1.8
	50	23.7 ± 1.6
	0	29.7 ± 1.3
4	2	31.5 ± 1.5
	5	23.8 ± 2.0
	10	32.9 ± 1.5
	0	35.1 ± 1.5
5	10	35.2 ± 2.7
	50	29.1 ± 1.6
	0	43.5 ± 2.3
6	5	30.1 ± 1.8
	10	25.6 ± 1.0
	50	32.9 ± 2.6
	0	36.4 ± 1.5
	53K antigen	
1	10	25.3 ± 1.8
	20	27.2 ± 1.5
	50	24.0 ± 2.0
	0	29.7 ± 1.1
2	10	27.9 ± 2.2
	30	21.6 ± 2.0
	50	27.4 ± 1.0
	0	32.3 ± 1.3
3	20	20.8 ± 1.7
	30	24.7 ± 0.7
	50	17.9 ± 2.0
	0	30.6 ± 1.5
4	30	22.6 ± 1.8
	50	32.8 ± 1.0
	0	35.4 ± 1.5

MAIRE A. SMITH
JOHN A. CLEGG
Department of Biochemical Parasitology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, England BR3 3BS

References and Notes

1. S. R. Smithers and M. J. Doenhoff, in *Immunology of Parasitic Infections*, S. Cohen and K. Warren, Eds. (Blackwell, Oxford, 1982), pp. 527–607.
2. A. E. Butterworth *et al.*, *Immunol. Rev.* **61**, 5 (1982); A. Capron, J. L. Dessaint, M. Capron, M. Joseph, G. Torpier, *ibid.*, p. 41.
3. D. A. Dean, *Exp. Parasitol.* **55**, 1 (1983).
4. J. A. Clegg and M. A. Smith, *Adv. Parasitol.* **16**, 165 (1978).
5. S. Horowitz, M. Smolarsky, R. Arnon, *Eur. J. Immunol.* **12**, 327 (1982).
6. M. Strand, A. McMillan, D. Pan, *Exp. Parasitol.* **54**, 145 (1982); C. P. Tavares *et al.*, *Z. Parasitenkd.* **70**, 189 (1984); C. Dissous, J. M. Grzych, A. Capron, *J. Immunol.* **129**, 2232 (1982).
7. M. Knight, A. J. G. Simpson, G. Payares, M. Chaudri, S. R. Smithers, *Eur. Mol. Biol. Org. J.* **3**, 213 (1984); D. W. Taylor, J. S. Cordingley, A. E. Butterworth, *Mol. Biochem. Parasitol.* **10**, 305 (1984).

8. M. A. Smith, J. A. Clegg, D. Snary, A. J. Trejdosiewicz, *Parasitology* **84**, 83 (1982); M. A. Smith, J. A. Clegg, D. Snary, *Trans. R. Soc. Trop. Med. Hyg.* **78**, 187 (1984).
9. D. A. Harn, M. Mitsuyama, J. R. David, *J. Exp. Med.* **159**, 1371 (1984).
10. W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83 (1974).
11. The experimental monkeys were given two intramuscular injections of antigen (200 μg in alhydrogel) at an interval of 4 weeks; control monkeys received adjuvant alone. Two weeks later all the monkeys were challenged with 1200 cercariae. After a further 8 weeks the hepatic portal system was perfused to recover adult schistosomes. From the three vaccinated monkeys 471, 557, and 380 schistosomes were recovered (mean, 469.3 ± 51.1); from the two control animals 830 and 783 schistosomes were recovered (mean, 801.5 ± 18.5) ($P < 0.01$; *t*-test).
12. S. L. James, P. C. Natovitz, W. L. Farrar, E. J. Leonard, *Infect. Immun.* **44**, 569 (1984).
13. A. Sher, R. Correa-Oliveira, S. Hieny, R. Hussain, *J. Immunol.* **131**, 1460 (1983).
14. Immunoglobulin M and subclasses of IgG were estimated in sera of mice taken just before challenge by radial immunodiffusion in gel (Meloy Laboratories, Springfield, Va.). Specific levels of IgE antibodies against the 155K antigen were measured in a solid-phase radioimmunoassay (*5*) with rabbit antibody to mouse IgE (Sera-Lab) affinity-purified against mouse IgE and labeled with ^{125}I.
15. Schistosome perfusion from the portal system was performed as described by S. R. Smithers and R. J. Terry [*Parasitology* **55**, 695 (1965)].
16. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
17. H. Towbin, T. Staehlin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
18. P. L. Ey, S. J. Prowse, C. R. Jenkin, *Immunochemistry* **15**, 429 (1978).
19. F. J. Ramalho-Pinto *et al.*, *Exp. Parasitol.* **34**, 360 (1974).
20. The expert technical assistance of F. Jones and R. Nelms is gratefully acknowledged.

19 September 1984; accepted 7 December 1984

Characterization of Long Terminal Repeat Sequences of HTLV-III

Abstract. *The nucleotide sequence of the long terminal repeat sequence (LTR) of the human T-cell leukemia (lymphotropic) virus type III (HTLV-III) was determined. This virus is associated etiologically with the acquired immune deficiency syndrome. The LTR was found to be 634 base pairs in length with U3, R, and U5 regions of 453, 98, and 83 bp, respectively. The proviral DNA is flanked by a 7-base-pair direct repeat. The promoter and polyadenylation signals are situated 27 and 24 base pairs upstream from the respective transcriptional initiation and polyadenylation sites. The primer binding site is complementary to transfer RNA-lysine. The LTR of HTLV-III, like that of HTLV-I, showed a limited homology to enhancer-like sequences within two genes expressed specifically in T lymphocytes, T-cell growth factor, and γ-interferon. Structural comparisons revealed that the LTR of HTLV-III is distantly related to those of HTLV-I, HTLV-II, and bovine leukemia virus.*

The human retrovirus termed HTLV-III has been etiologically linked to the acquired immune deficiency syndrome (AIDS) (*1–3*), a disease characterized by opportunistic infections and malignancies such as Kaposi's sarcoma. This syndrome is also characterized by a preferential loss of $OKT4^+$ lymphocytes (*4*), the principal cell target for HTLV-III replication (*2*). In a recent study (*3*), serum samples from 95 percent of patients with AIDS or AIDS-associated disorders reacted with HTLV-III viral proteins, whereas samples from 30 percent of normal homosexuals and less than 1 percent of normal heterosexuals showed such a reaction (*3*). Like the human T-cell leukemia (lymphotropic) virus types I and II, HTLV-III is lymphotropic; shows a particular capacity to infect T4 lymphocytes; contains a relatively large DNA polymerase (about 90,000 daltons), which is Mg^{2+} dependent; contains a major core protein of 24,000 daltons; induces formation of giant multinucleated cells; and, like HTLV-I and HTLV-II, contains a novel gene at the 3′ end of the viral genome referred to as the long open reading frame (LOR) (*5*). Among animal retroviruses, bovine leukemia virus (BLV) also shares many of these characteristics as well as actual protein sequence homologies with HTLV-I (*6*). HTLV-III also shows substantial genomic homology with visna virus (*7*), a retrovirus that causes a chronic neurological disease of sheep.

The availability of recombinant DNA clones of HTLV-III prepared in our laboratory (*8, 9*) has enabled us to study the molecular details of this virus genome. The LTR region of HTLV-III was first analyzed since this region in other retroviruses is known to include regulatory sequences for viral transcription, host cell tropism, and determination of pathogenetic capabilities (*10*). Furthermore, comparison of the HTLV-III LTR with the LTR's of HTLV-I, HTLV-II, BLV, and other retroviruses, especially members of the subfamily Lentiviridae, may provide evidence of the evolutionary origin of this virus.

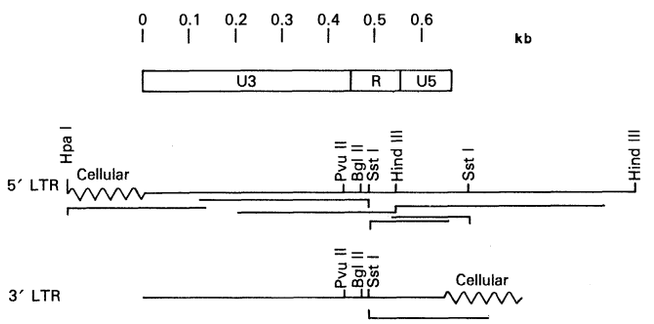

Fig. 1 (left). A schematic drawing of HTLV-III LTR, restriction map of the 5′ and 3′ LTR regions of the HXB2 (*9*) clone and sequencing strategy. To determine the cell-LTR boundaries we compared the sequencing data from an unintegrated DNA clone BH8 (*8*), corresponding to the 3′ 3.5-kb portion of the viral genome, with the sequence of HXB2. After restriction enzyme digestions, the labeling of 5′ ends was accomplished with γ-^{32}P-labeled adenosine triphosphate and the T4 polynucleotide kinase; 3′ ends were labeled with α-^{32}P dideoxy-ATP and terminal deoxynucleotidal transferase. The sequence was determined by the method of Maxam and Gilbert (*22*). The U3-R boundary was localized by S1 nuclease mapping (*23*) (see Fig. 2) and strong-stop cDNA synthesis. The R-U5 boundary was determined from the sequence of a cDNA clone obtained by using oligo(dT) as primer and viral RNA as template (not shown).

```
                                                                TAGTAGT
U3
TGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACA    60
--
CACAAGGCTACTTCCCTGATTAGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCAC   120

TGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCA  180

ATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGGATGGATGACCCGG  240

AGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCACGTGGCCCGAG  300

AGCTGCATCCGGAGTACTTCAAGAACTGCTGATATCGAGCTTGCTACAAGGGACTTTCCG  360

CTGGGGACTTTCCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGAT  420

      TATA  Pvu II          U3 ▼ R                Bgl  II
CCTGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGA  480
      ------------
                                                      Hind  III
       SstI                                          Poly(A) s
GCCTGGGAGCTCTCTGGCTAGCTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCT  540
                                                     ----------
      R  ▼ U5
TGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTC  600

                                  U5 ▼     tRNA Lys
AGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGAC         660
                                  -- ------------------
                                     TAGTAGT
```

Fig. 2 (right). Complete HTLV-III LTR sequence of the genomic clone obtained from the HTLV-III–producing T-cell line H9 (*2*).