mechanism may be relevant to AIDS because HTLV-III isolates have shown a range of genomic variability, especially in the envelope region[22], and a relatedness of HTLV-III to visna virus was demonstrated recently[23]. It will be necessary to obtain serial isolates and serum samples from individuals throughout the course of the disease to determine whether HTLV-III neutralizing antibodies exert selective pressure on viral mutants.

The presence of HTLV-III neutralizing antibodies in sera of individuals exposed to HTLV-III demonstrates an immunological response during the course of disease development which may be used therapeutically. Furthermore, it gives hope that appropriate vaccine approaches may be effective in preventing viral infection. Further studies will determine whether virus neutralizing antibodies in the sera of patients have prognostic value or will be indicative of appropriate treatment regimes.

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An anti-idiotype vaccine against experimental schistosomiasis

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Schistosomiasis is a parasitic infection of man which is widespread in tropical countries, and which so far has resisted attempts at control. We have been approaching the problem from an immunological angle. We have previously reported[1] the production of a rat monoclonal IgG2a antibody against *Schistosoma mansoni* which exhibits marked cytoxicity for schistosomula in the presence of eosinophils and a high degree of protection by passive transfer in naive rats. This antibody, IPLSm1, was shown to bind specifically to a schistosomulum membrane target antigen defined as a glycoprotein of relative molecular mass 38,000 (38K)[2], which is strongly immunogenic in schistosome infection of various animal species including man[3]. Although theoretically the 38K protein represents an excellent candidate for a potential vaccine against schistosomiasis, the glycanic nature of the epitope recognized by IPLSm1 limits its production by DNA recombinant technology. It was, moreover, shown that, together with protective antibodies, the 38K molecule was able to induce the production of blocking IgG2c antibodies that inhibit the functional properties of IPLSm1 both *in vitro* and *in vivo*[4]. Therefore, following Jerne's network theory[5], we considered an alternative approach, the possibility of immunization using anti-idiotype antibodies. In the present study, rat monoclonal anti-idiotype antibodies were produced against IPLSm1 (AB_1). Anti-idiotype antibodies (AB_2) were selected by their capacity to inhibit the binding of radioiodinated AB_1 to its 38K target antigen. Sera from naive LOU rats immunized with a purified AB_2 preparation contained specific anti-schistosome antibodies (AB_3) which bound to 38K. AB_3 antibodies were strongly cytotoxic for schistosomula in the presence of rat eosinophils and conferred highly significant protection by passive transfer. Most importantly, rats immunized with AB_2 demonstrated marked protection (50–80%) to a challenge infection.

Monoclonal antibodies to IPLSm1 were obtained in a homologous hybridization system. Male LOU rats were injected subcutaneously with 1 mg of purified IPLSm1 plus 1 mg of glutaraldehyde-aggregated human IgG in the presence of complete Freund's adjuvant. Two weeks later, the rats received a second injection of the same preparation. The immunization was completed by two subcutaneous injections of 1 mg of

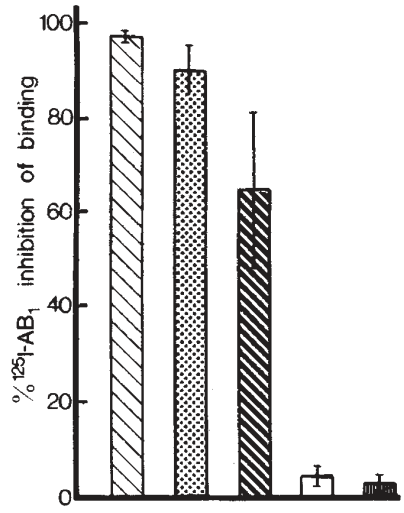

Fig. 1 Inhibition of radiolabelled AB_1 antibody (IPLSm1 IgG2a antibody) to the 38K antigen. Experiments were performed on PVC microtitre plates precoated with C_3-109 IgM antibody (an *S. mansoni* rat monoclonal antibody which recognizes the 38K molecule but does not interfere with IPLSm1 binding). Each well of the PVC plates was coated with 100 μl of a 10 μg ml^{-1} solution of C_3-109 IgM diluted with 10 mM phosphate-buffered saline (PBS), a procedure which gives better fixation of the 38K antigen. After 2 h, the plates were washed twice in PBS buffer and saturated for 30 min with 200 μl of a 2% bovine serum albumin (BSA) solution in PBS. The plates were then washed twice in PBS–0.1% BSA. A Nonidet P-40 extract of schistosomula was added (100 μl antigen solution containing 100 μg of protein). After 2 h exposure at 37 °C, plates were washed twice in PBS–0.1% BSA buffer. For the test, 50 μl of ^{125}I-labelled IPLSm1 IgG2a was incubated with 50 μl of sera at a dilution of 1/25 in PBS-0.1% BSA buffer for 1 h at 37 °C and for 16 h at 4 °C and the plates were then washed three times in PBS-0.1% BSA buffer. The wells were counted in a γ-counter. The percentage of IPLSm1 inhibition binding was calculated using the following formula: $a - b/a \times 100 = \%$ of inhibition, where a = c.p.m. obtained when ^{125}I-AB_1 was incubated with 50 μl of PBS-0.1 % BSA buffer, and b, c.p.m. obtained when ^{125}I-AB_1 was incubated with 50 μl serum from a rat bearing a subcutaneous IPLSm1 hybridoma (▨), 50 μl serum from a rat immunized with AB_2 (JM8-36) (▩), 50 μl serum from a rat immunized with normal IgM (□), 50 μl serum from a 4-week-infected rat (▩) or 50 μl of normal rat serum (▥) (mean of five duplicate experiments ± s.d.).

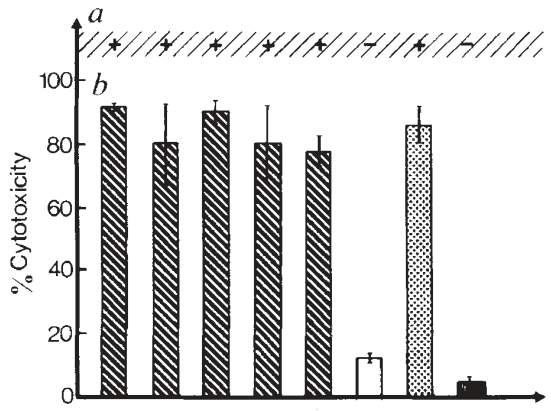

Fig. 2 Humoral response to AB_2 immunization. *a*, Immunofluorescence tests; *b*, eosinophil-dependent cytotoxicity. Indirect immunofluorescence tests were performed[1] on deep-frozen sections of schistosomula (8 μm). *S. mansoni* schistosomula were prepared by penetration of cercariae through abdominal mouse skin. Sera (tested at a dilution of 1/20 in 10 mM PBS) were collected from LOU/M rats immunized by JM8-36 (AB_2) antibody (▨) purified from ascitic fluid as described previously[15]. A 1-mg aliquot of the purified preparation in physiological saline was injected subcutaneously at 2-week intervals. Control sera were obtained from rats injected in the same conditions with the IgM fraction of normal rat serum (□). Eosinophil-dependent cytotoxicity was measured after 48 h contact of skin schistosomula, preincubated overnight with the sera at a final dilution of 1/16, with a rat eosinophil-rich population (40–60% eosinophils). The percentage cytotoxicity was compared at equivalent dilutions with control sera, normal rat serum (▥) or serum from rats infected for 4 weeks with *S. mansoni* (▨). All results correspond to a mean of two duplicate experiments ± s.d.

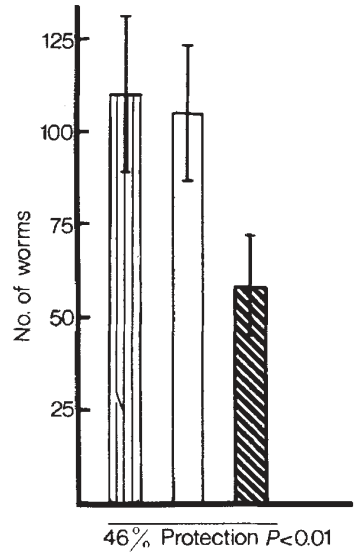

Fig. 3 Passive transfer of AB_3 antibodies. Serum (1 ml) collected from JM8-36 immunized rats (▨) or from rats immunized with normal IgM (□) was injected intravenously into LOU rats infected 4 h previously with 800 *S. mansoni* cercariae. Parasitic burdens were evaluated 3 weeks later by liver perfusion[16]. The number of worms obtained from these groups was compared with the parasite burden of rats injected with 1 ml of physiological saline (▥). Percentage protection was calculated by the formula $(a-b)/a \times 100$, where a = the number of worms recovered from the saline-injected control group and b = the number of worms recovered from rat injected with 1 ml of AB_3 serum.

IPLSm1 and 1 mg of aggregated human IgG in incomplete Freund's adjuvant at 2-week intervals. The Ouchterlony test was used to select rats showing the highest responses and spleen cells from these animals were fused with cells from the IR983F rat myeloma cell line[6] as described previously[1]. The hydrid cell supernatants were screened for their ability to inhibit the binding of ^{125}I-labelled IPLSm1 to the 38K target antigen coated on a polyvinyl chloride (PVC) plate. From 200 hybrid cell supernatants obtained from two successive cell fusion experiments, we selected 29 supernatants inducing significant levels of inhibition (>70%).

Analysis of the immunological components of these hybrid cell supernatants by the Ouchterlony test revealed that in most cases (23/29) they contained antibodies of the IgM class. The inhibitory activity of hybrid cell supernatants on IPLSm1 binding to 38K strongly suggested that the antibodies produced were able to fix to an epitope of IPLSm1 which was close to or part of its antigen-combining site, indicating that AB_2 were paratope-induced antibodies bearing an internal image of the original epitope.

We next investigated the potential use of such antibodies to induce active immunization in naive LOU rats. Two questions in particular were studied: Is it possible to use AB_2 antibodies to induce the production of AB_3 antibodies which will reproduce the effector mechanism described previously *in vitro* both in experimental schistosomiasis[7] and with AB_1 antibody, involving eosinophil-mediated cytotoxicity[1] ? Does this immunization lead to a significant protection of naive rats against a challenge infection?

To answer the first question, we followed the kinetics of the antibody response in naive LOU rats immunized with AB_2 antibodies. Successive injections of purified IgM anti-idiotype antibody (JM8-36) for 4 weeks elicited anti-schistosome antibodies (AB_3) which were detected by indirect immunofluorescence on cryopreserved *S. mansoni* schistosomulum sections. In all cases, the fluorescence reaction was observed at the surface level. These observations, suggesting a close correlation between the structure of the anti-idiotype antibody and an epitope present on the surface of *S. mansoni* schistosomula, were confirmed by competition of AB_1 by AB_3 on radiolabelled membrane preparations of schistosomula. AB3 preparations strongly inhibited the binding of AB_1 to the 38K molecule, indicating a close specificity for the same epitope (Fig. 1).

We explored the biological activity of these AB_3 antibodies induced by anti-idiotype immunization by determining their possible participation in eosinophil-dependent cytotoxicity reactions against *S. mansoni* schistosomula. A significant level of cytotoxicity (70–90%) was observed, close to that mediated by a 4-week *S. mansoni*-infected rat serum (Fig. 2) or by the AB_1 monoclonal antibody itself[1].

The relevance of these *in vitro* findings was fully supported by the demonstration that passive transfer of AB_3 antibodies to naive rats resulted in a significant protection of these animals to a challenge infection by *S. mansoni* cercariae (Fig. 3).

Finally, we studied the direct protective effect of an anti-idiotype immunization. In two series of experiments involving 30 naive LOU rats, immunization with AB_2 resulted in 50–76% protection to a challenge infection (Fig. 4).

The work presented here clearly demonstrates that immunization with an anti-idiotype monoclonal antibody can reproduce several parameters of the acquired immunity observed in experimental rat schistosomiasis. First, immunization with AB_2 resulted in the production of specific anti-*S. mansoni* antibodies in animals which had never been exposed to the parasite antigen. The antibodies produced exhibited the same specificity for the 38K molecule as the original AB_1 monoclonal antibody used to produce the anti-idiotype antibody, suggesting that the AB_2 produced may represent an internal image of the original epitope. However, most importantly, such experiments raise the possibility of inducing a strong immunity against schistosomes, as shown by both active immunization and passive transfer experiments.

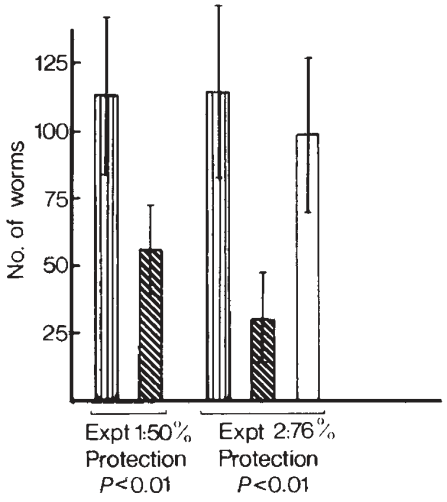

Fig. 4 Protective effect of JM8-36 immunization. LOU rats immunized as described in Fig. 2 legend, giving positive immunofluorescence reactions and significant eosinophil-dependent cytotoxicity, were infected with 800 *S. mansoni* cercariae. Parasite burdens were measured 3 weeks later by liver perfusion[16]. The number of worms obtained from rats immunized with JM8-36 AB_2 antibodies (▨) was compared with those obtained from control groups, that is LOU rats injected with physiological saline (▥) or with IgM purified from normal rat serum (□). The percentage of protection was calculated by the formula $(a-b)/a \times 100$, where a = the number of worms obtained from the saline-injected control group and b = the number of worms recovered from AB_2-immunized rats.

Thus, immunization with anti-idiotype antibodies represents an alternative approach to immunization against pathogens. Although recent studies have produced encouraging results[8-12] concerning the potential substitution of conventional vaccines by anti-idiotype antibodies, this strategy is only in its early stages[13]. In the context of schistosomiasis, idiotype vaccines could be of particular use when relevant protective epitopes cannot easily be produced by the modern tools of molecular biology. Although the rat is a non-permissive host, there is now clear evidence[14] that all the specific effector mechanisms of immunity described in this model also occur in human infection. The possibility of using such immunization procedures in humans remains unexplored and cannot be directly extrapolated from experimental infections. However, work in progress in our laboratory, indicating the existence of cross-reacting idiotypes in human schistosome infection, is encouraging.

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Individual-specific 'fingerprints' of human DNA

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Simple tandem-repetitive regions of DNA (or 'minisatellites') which are dispersed in the human genome frequently show substantial length polymorphism arising from unequal exchanges which alter the number of short tandem repeats in a minisatellite[1-4]. We have shown previously that the repeat elements in a subset of human minisatellites share a common 10–15-base-pair (bp) 'core' sequence which might act as a recombination signal in the generation of these hypervariable regions[5]. A hybridization probe consisting of the core repeated in tandem can detect many highly polymorphic minisatellites simultaneously to provide a set of genetic markers of general use in human linkage analysis[5]. We now show that other variant (core)$_n$ probes can detect additional sets of hypervariable minisatellites to produce somatically stable DNA 'fingerprints' which are completely specific to an individual (or to his or her identical twin) and can be applied directly to problems of human identification, including parenthood testing.

Three human minisatellites, termed 33.5, 33.6 and 33.15, each comprised of tandem repeats of various versions of the core sequence, have been cloned previously and characterized[5] (Fig.

Table 1 Similarities of DNA fingerprints between random pairs of individuals

Probe	DNA fragment size (kb)	No. of fragments per individual ±s.d.	Probability x that fragment in A is present in B	Maximum mean allelic frequency/ homozygosity
33.6	10-20	2.8 ± 1.0	0.11	0.06
	6-10	5.1 ± 1.3	0.18	0.09
	4-6	5.9 ± 1.6	0.28	0.14
33.15	10-20	2.9 ± 1.0	0.08	0.04
	6-10	5.1 ± 1.1	0.20	0.10
	4-6	6.7 ± 1.2	0.27	0.14

Samples (8 μg) of blood DNA[6] taken from a random sample of 20 unrelated British caucasians were digested with *Hin*fI and Southern blot hybridized with minisatellite probes 33.6 or 33.15 as described in Fig. 1 legend. Each DNA fingerprint (individual A) was compared with the pattern in the adjacent gel track (individual B), and the number of bands in A which were clearly absent from B, plus those which had a co-migrating counterpart of roughly similar autoradiographic intensity in B, were scored. The data shown are averages for all pairwise comparisons. A small proportion (~6%) of additional weakly hybridizing fragments in A were matched by strongly hybridizing fragments in B, and because in such cases it was not possible to decide whether the band in A was also present in B, such fragments were ignored. If co-migrating bands in A and B are always identical alleles of the same minisatellite locus, then the probability x that an allele in A is also present in B is related to the frequency q of that allele by $x = 2q - q^2$. As the allele frequency is low, then $q^2 \ll q$ and therefore the mean probability $\hat{x}$ is approximately related to the mean allele frequency $\hat{q}$ by $\hat{x} \simeq 2\hat{q}$. Furthermore, assuming that there is little variance in q between alleles, then the number of alleles $n \simeq 1/\hat{q}$ and the mean homozygosity is therefore approximately given by $\sum_1^n q_i^2 \simeq n\hat{q}^2 = \hat{q}$. In practice, an (unknown) proportion of co-migrating bands in A and B will be derived by chance from different minisatellite loci, and thus the estimates of mean allele frequency and homozygosity are maximal and depend on the electrophoretic resolution of minisatellite fragments. Probability estimates: the mean probability that all fragments detected by probe 33.15 in individual A are also present in B is $0.08^{2.9} \times 0.20^{5.1} \times 0.27^{6.7} = 3 \times 10^{-11}$.