4. P. D. Ling, J. J. Ryon, S. D. Hayward, *J. Virol.* **67**, 2990 (1993).
5. P. D. Ling, D. R. Rawlins, S. D. Hayward, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 9237 (1993).
6. U. Zimber-Strobl *et al.*, *EMBO J.* **12**, 167 (1993).
7. T. Henkel, P. D. Ling, S. D. Hayward, M. G. Peterson, *Science* **265**, 92 (1994).
8. S. R. Grossman, E. Johannsen, X. Tong, R. Yalamanchili, E. Kieff, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7568 (1994); N. J. Matsunami *et al.*, *Nature* **342**, 934 (1989).
9. P. D. Ling, J. J.-D. Hsieh, I. K. Ruf, D. R. Rawlins, S. D. Hayward, *J. Virol.* **68**, 5375 (1994).
10. T. Tun *et al.*, *Nucleic Acids Res.* **22**, 965 (1994).
11. S. Dou *et al.*, *Mol. Cell. Biol.* **14**, 3310 (1994).
12. S. L. Madden *et al.*, *Science* **253**, 1550 (1991).
13. Y. Shi, E. Seto, L. S. Chang, T. Shenk, *Cell* **67**, 377 (1991).
14. A. L. Gashler, S. Swaminathan, V. P. Sukhatme, *Mol. Cell. Biol.* **13**, 4556 (1993); I. G. Cowell, *Trends Biochem. Sci.* **19**, 38 (1994).
15. Abbreviations for the amino acid residues are as follows: A, Ala; E, Glu; F, Phe; H, His; I, Ile; K, Lys; N, Asn; Q, Gln; R, Arg; S, Ser; T, Thr; and W, Trp.
16. J. J.-D. Hsieh and S. D. Hayward, unpublished data.
17. S. Natesan and M. Z. Gilman, *Genes Dev.* **7**, 2497 (1993).
18. R. Amakawa *et al.*, *Genomics* **17**, 306 (1993).
19. T. Furukawa, S. Maruyama, M. Kawaichi, T. Honjo, *Cell* **69**, 1191 (1992).
20. F. Schweisguth and J. W. Posakony, *ibid.*, p. 1199.
21. M. E. Fortini and S. Artavanis-Tsakonas, *ibid.* **79**, 273 (1994).
22. L. W. Ellisen *et al.*, *ibid.* **66**, 649 (1991).
23. We acknowledge the contribution of P. Ling in laying the foundation for this work and J. Tan for technical assistance. Supported by grants from NIH (CA42245) and the American Cancer Society (FRA 429).

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Expression Cloning of a Protective *Leishmania* Antigen

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Parasite-specific CD4$^+$ T cells have been shown to transfer protection against *Leishmania major* in susceptible BALB/c mice. An epitope-tagged expression library was used to identify the antigen recognized by a protective CD4$^+$ T cell clone. The expression library allowed recombinant proteins made in bacteria to be captured by macrophages for presentation to T cells restricted to major histocompatibility complex class II. A conserved 36-kilodalton member of the tryptophan–aspartic acid repeat family of proteins was identified that was expressed in both stages of the parasite life cycle. A 24-kilodalton portion of this antigen protected susceptible mice when administered as a vaccine with interleukin-12 before infection.

The immunology of *Leishmania major* infection has been characterized in inbred strains of mice. The response is CD4-dependent, presumably reflecting the residence of the obligate intracellular amastigotes in macrophages within endolysosomal compartments that contain major histocompatibility complex (MHC) class II molecules (*1*). Thus, mice with disruption of the MHC class II or β_2-microglobulin genes are, respectively, susceptible or resistant to primary *L. major* infection, reflecting requirements for CD4$^+$ but not CD8$^+$ T cells (*2*). Most mice control an infection in association with the development of a type 1 T helper (T_H1) cell response that ensures production of the macrophage-activating cytokine interferon γ (IFN-γ), which is required for cure (*3*). In contrast, susceptible BALB/c mice develop a T_H2 cell response that is incapable of mediating parasite clearance and that interferes with the actions of T_H1-derived cytokines, primarily through the production of interleukin-4 (IL-4) (*4*).

The importance of T_H1 cells for the development of protective immunity against infection with *L. major* has been confirmed by means of clonally derived parasite-specific T cells. After adoptive transfer, a number of parasite-specific CD4$^+$ T_H1 cell clones and lines protected sublethally irradiated BALB/c mice (*5*, *6*). Although protective T cell clones belong to the T_H1 subset, some parasite-specific T_H1 T cell clones are not protective and even exacerbate the disease (*7*). Taken together, these results suggest that the ability of T cells to eliminate *L. major* from the infected host depends both on the cells' fine specificity and the type of cytokines that they secrete after antigen stimulation.

To identify antigens capable of eliciting this protective T cell response, we identified the parasite antigen recognized by the protective T cell clone 9.1-2, a T_H1 clone derived from spleen of BALB/c mice that had been vaccinated with an antigenic fraction separated from a soluble extract of *L. major* promastigotes (*6*). This T cell clone uses a heterodimeric $V_\beta4$, $V_\alpha8$ T cell antigen receptor (TCR) representative of the clonotypic, restricted TCR expansion that occurs early after the infection of mice with *L. major* (*8*). We used *L. major* promastigote mRNA to construct a complementary DNA (cDNA) library in prokaryotic expression vector pET3a-δ9 (*9*). This vector was designed for the high-level expression of cloned cDNA molecules in *Escherichia coli* as fusion proteins containing an epitope from influenza hemagglutinin (HA); this epitope is recognized by monoclonal antibody (mAb) 12CA5 (*10*). Pools of 5000 transformants were grown, and the expression of recombinant proteins was induced after infection with a recombinant λ bacteriophage carrying the T7 DNA polymerase gene. The recombinant proteins were purified from crude bacterial lysates by means of affinity columns and dialysis against isotonic buffer (*11*).

We incubated bone marrow–derived macrophages from BALB/c mice (*12*) in the presence of pools of recombinant proteins with mAb 12CA5 to facilitate targeting to MHC class II compartments through Fc receptor (FcR)–mediated internalization. Subsequently, LMR 16.2 T cell hybridoma cells, which were derived from the parasite-specific T_H1 clone 9.1-2 (*13*), were added, and the supernatants were screened for IL-2 secretion as an index of T cell activation. Three pools from ~150,000 cDNA clones induced IL-2 secretion that could be abolished by the addition of mAbs to I-A^d. Antigen-presenting cells from mismatched MHC class II mice (C57BL/6, CBA) did not stimulate IL-2 secretion from LMR 16.2 in this assay. One of the three pools was sequentially fractionated and rescreened until a single colony (clone 23.12.10.33) that gave rise to IL-2 secretion by hybridoma LMR 16.2 was identified.

The recombinant protein expressed by this clone had a molecular size of 24 kD. Incubation of the purified protein with LMR 16.2 or clone 9.1-2 resulted in the release of IL-2 or IFN-γ, respectively, in a dose-dependent manner (Fig. 1). Production of IFN-γ was inhibited by incubation with mAbs to either I-A^d or CD4 (*14*). No IFN-γ was generated when cells were incubated with recombinant ovalbumin that had been fused with the HA epitope and produced in *E. coli* by the same method (*14*).

The 950–base pair (bp) cDNA insert from clone 23.12.10.33 hybridized to two *L. major* transcripts of 1500 and 1800 nucleotides as revealed by Northern (RNA) blot-

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ting. Similar results were obtained with RNA prepared from related *Leishmania* species, including *L. donovani*, *L. amazonensis*, and *L. chagasi* (Fig. 2A) (*15*). Using polymerase chain reaction (PCR) technology and RNA prepared from *L. major* promastigotes, we isolated a full-length cDNA clone containing an open reading frame of 312 amino acids (Fig. 3). The predicted 36-kD protein exhibited homology with intracellular receptors for activated protein kinase C, or RACKs, that are members of an ancient family of regulatory proteins containing regularly spaced Trp-Asp (WD) amino acid sequence motifs (*16*). Cloning of the corresponding cDNA from *L. chagasi* promastigotes revealed 96% identity between the two related *Leishmania* species, suggesting that this protein is highly conserved (Fig. 3). Finally, antiserum raised against the 24-kD protein expressed by clone 23.12.10.33 reacted with a 36-kD protein in both *L. major* and *L. amazonensis* promastigote and amastigote lysates as assessed by protein immunoblotting, indicating that this antigen, which we designated LACK (*Leishmania* homolog of receptors for activated C kinase), was expressed in both stages of the parasite (Fig. 2B).

The identification of a potential target of the early immunodominant response by $CD4^+$ T cells that use the $V_\beta 4$, $V_\alpha 8$ TCR (*8*) suggested that vaccination in a manner that could stably induce T_H1 cell differentiation might provide protection against *Leishmania* infection. Stimulation during subsequent parasite challenge could provide inhibitory signals for T_H2 cell differentiation in response to parasite antigens presented later in infection. IL-12 has proven effective when administered as an adjuvant with uncharacterized soluble *Leishmania* antigens (SLAs) in immunizing susceptible BALB/c mice (*17*), but protection by a recombinant protein plus IL-12 has not been demonstrated. BALB/c mice were immunized in the right footpad twice with 25 μg of recombinant p24 expressed from clone 23.12.10.33 and 1 μg of recombinant murine IL-12 at 4 and 2 weeks before infection as described (*17*). Additional groups of mice received antigen p24 alone or recombinant ovalbumin expressed as a fusion protein with the HA epitope with IL-12. Recombinant *L. major* gp63, an abundant protease expressed by all *Leishmania* species (*18*), was also tested

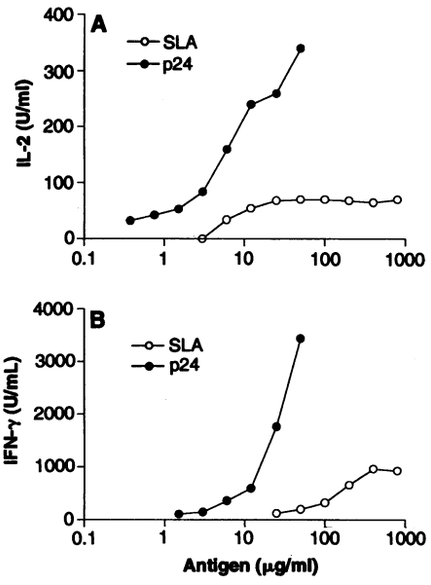

Fig. 1. The 24-kD recombinant protein expressed by clone 23.12.10.33 triggers the secretion of (**A**) IL-2 by LMR 16.2 T cell hybridoma cells and (**B**) IFN-γ by clone 9.1-2 T cells. Fifty thousand LMR 16.2 or 9.1-2 cells per well were plated in 96-well tissue culture plates with 10^6 BALB/c splenocytes and varying doses of p24 recombinant protein or soluble *L. major* antigen (SLA) [prepared as described in (*6*)] in DMEM containing glucose (4.5 mg/ml; Gibco-BRL), 10% FCS, 2 mM glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and 5×10^{-5} M β-mercaptoethanol. Supernatants were harvested at (A) 24 or (B) 48 hours and were immediately assayed for IL-2 or IFN-γ activity with the IL-2–dependent cell line CTLL-2 or a specific two-site ELISA, respectively. The concentrations of IL-2 and IFN-γ were calculated by comparison with standard curves generated with recombinant human IL-2 or murine IFN-γ (Gibco-BRL).

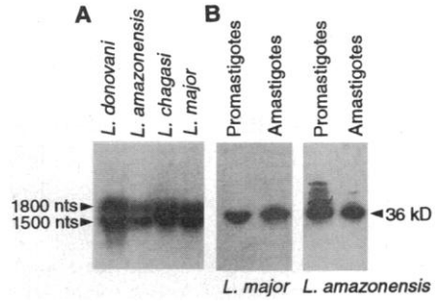

Fig. 2. The cDNA insert from clone 23.12.10.33 hybridized to two *Leishmania* transcripts and corresponds to a 36-kD protein that is expressed in both promastigotes and amastigotes. (**A**) RNA blot analysis. Polyadenylated RNA (2 μg) from *L. major*, *L. chagasi*, *L. amazonensis*, and *L. donovani* promastigotes (*15*) was loaded in a 1% formaldehyde agarose gel. After electrophoresis, the RNA was blotted onto Hybond-N (Amersham), and the filter was hybridized at 42°C with the nick-translated insert from clone 23.12.10.33 (5×10^5 cpm/ml). The filter was washed at 65°C in 2× saline sodium citrate (SSC); nts, nucleotides. (**B**) Protein immunoblot analysis. Lysates were prepared from *L. major* and *L. amazonensis* promastigotes and amastigotes and analyzed by immunoblotting with a rabbit polyclonal antiserum against the protein expressed by clone 23.12.10.33.

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p36major    1 MNYE----GHLKGHRGWVTSLACPQQAGSYIKVVSTSRDGT
p36chag.    1 MNYE----GHLKGHRGWVTSLACPQQAGSYIKVVSTSRDGT
RACK        1 MTEQMTLRGTLKGHNGWVTQIATTPQFPD--MILSASRDKT

p36major   38 VISWKANPDRHSWTATTVCRTTASRGTPASCRACRWATPPY
p36chag.   38 AISWKANPDRHSVTATTVCRATASRGTPASCRVCRWATPPY
RACK       40 IIMWKLTRDETNYGIP----QRALRGHSHFVSDVVISSDGQ

p36major   79 YALTVSWDRSIRMWDLRIGQCQRKFLKHTKDVLTVAFSPDD
p36chag.   79 YALTVSWDRSIRMWDLRIGQCQRKFLKHTKDVLAVALSPDD
RACK       77 FALSGSWDGTLRLWDLTTGTTTRRFVGHTKDVLSVAFSSDN

p36major  120 RLIVSAGRDNVIRVWNVAGECMHEFLRDGHEDWVSSICFSP
p36chag.  120 RLIVSAGRDNVIRVWNVAGECMHEFLRDGHEDWVSSICFSP
RACK      118 RQIVSGSRDKTIKLWNTLGVCKYTVQDESHSEWVSCVRFSP

p36major  161 SLEHPIVVSGSWDNTIKVWNVNGGKCERTLKGHSNYVSTVT
p36chag.  161 SLEHPIVVSGSWDNTIKVWNVNGGKCERTLKGHSNYVSTVT
RACK      159 NSSNPIIVSCGWDKLVKVWNLANCKLKTNHIGHTGYLNTVT

p36major  202 VSPDGSLCASCGKDGAVLLWDLSTGEQLFKINVESAINQIG
p36chag.  202 VSPDGSLCVSGGKDGAVLMWDLSTGEQLFKINVESTINQIA
RACK      200 VSPDGSLCASGGKDGQAMLWDLNEGKHLYTLDGGDIINALC

p36major  243 FSPNRFWMCVATERSLSVYDLESKAVIAELTPDG------A
p36chag.  243 FSSNRFWMCVATERSLSVYDLESKAVIAELTPDG------A
RACK      241 FSPNRYWLCAATGPSIKIWDLEGKIMVDELKQEVISTSSKA

p36major  278 KPSECISIAWSADGNTLYSGHKDNLIRVWSIS-DAE
p36chag.  278 KPSECISIAWSADGNTLYSGHKDNLIRVWSIS-DAE
RACK      282 EPPQCTSLAWSADGQTLFAGYTDNLVRVWQVTIGTR
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Fig. 3. The 36-kD protein is highly conserved among related *Leishmania* species and exhibits homology with intracellular receptors for activated protein kinase C (RACKs). The deduced amino acid sequences of *L. major* and *L. chagasi* 36-kD proteins were aligned and compared with the RACK1 sequence (*16*). Residues identical or similar are boxed in gray. Identical residues are in bold. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Dashes indicate spacing for optimal sequence alignment.

with IL-12. This antigen did not react with a panel of *L. major*–specific hybridomas that expressed the dominant $V_\beta 4$, $V_\alpha 8$ TCR expanded early after infection (*19*). Control groups included untreated mice, mice that received IL-12 alone at the times of immunization, and mice that received antibody to IL-4 (anti–IL-4) at the time of infection to induce healing (*4*). After challenge with 2×10^5 metacyclic promastigotes [purified as described in (*20*)] of *L. major*, we followed the course of the disease by measuring the size of the local lesion with a metric caliper (Fig. 4).

As compared with untreated animals, mice that received p24 plus IL-12 were significantly protected from disease, as assessed both by lesion size after 6 weeks [mean ± SEM, 4.7 ± 0.4 mm versus 2.6 ± 0.3 mm for untreated and vaccinated animals, respectively; probability (P) < 0.001] and by recovery of parasites through quantitative culture of tissues (mean parasite burden of 10^7 and 10^3 in untreated animals versus 10^3 and 10^1 promastigotes per 1 ml of homogenate in treated animals in footpad or spleen, respectively; $P < 0.01$ for both tissues). Protection under these conditions was bimodal, with 72% of mice in two experiments protected comparably to mice immunized with SLAs plus IL-12 or treated with anti–IL-4; the remaining 28% of animals had slow but progressive local swelling, although lesions were significantly smaller (3.4 ± 0.2 mm at 6 weeks) than in untreated mice. Approximately half of such animals maintained for prolonged periods (>3 months) continued to control parasite replication, as assessed both by the size of the footpad lesion and by the recovery of parasites measured by quantitative tissue culture. Animals that received p24 without IL-12, recombinant gp63 with IL-12, recombinant ovalbumin-HA fusion protein with IL-12, or IL-12 alone resembled untreated mice during the subsequent course of the disease. Optimal protection was achieved with anti–IL-4 or with SLAs with IL-12, consistent with the ability of these vaccination protocols to effect priming of greater numbers of T cells.

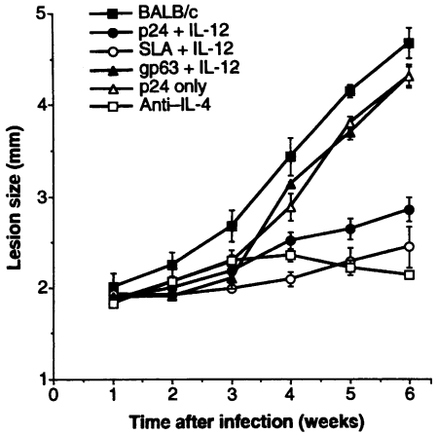

Fig. 4. BALB/c mice immunized with the p24 recombinant protein and IL-12 are protected against infection with *L. major*. Groups of 4 to 10 BALB/c mice were infected in the hind footpads with metacyclic promastigotes of *L. major* after no prior treatment (BALB/c); or vaccination twice, once at 4 weeks and once at 2 weeks, before infection with the antigens listed with or without concomitant IL-12; or a single dose of anti–IL-4 at the time of infection. The size of the local lesions was monitored weekly with a metric caliper. One of three representative experiments is shown. Symbols represent mean ± SEM. Abbreviations are as follows: p24, recombinant *L. major*–HA fusion protein; SLA, soluble *Leishmania* antigens; and gp63, recombinant *L. major* gp63 protease.

Other reports have documented that immunologic manipulations that enable BALB/c mice to control *L. major* infection are associated with the down-regulation of IL-4 expression (*4*, *21*). Analysis of popliteal lymph node cells from mice vaccinated with p24 plus IL-12 showed a diminution of IL-4 transcripts as compared with untreated animals or animals vaccinated with p24 alone (Fig. 5). Additionally, mice given p24 plus IL-12 showed an up-regulation of IFN-γ transcripts. Similarly, as assessed by an ELISPOT assay (*22*) to quantitate the numbers of spontaneous IL-4–secreting cells, we found that mice vaccinated with p24 plus IL-12 had down-regulated the numbers of IL-4–producing cells in the draining lymph nodes 6 weeks after infection (18 ± 4 per 10^5 cells) as compared with untreated mice (67 ± 7 per 10^5 cells) or mice vaccinated with p24 alone (54 ± 6 per 10^5 cells). Quantitation of total serum immunoglobulin E (IgE) concentrations by enzyme-linked immunosorbent assay (ELISA) demonstrated significant reduction in these mice (untreated mice, 14,000 ± 2800 ng/ml; p24 + IL-12–treated mice, 1200 ± 400 ng/ml; $P < 0.01$), consistent with the down-regulation of IL-4 production in vivo. Thus, immunization with this recombinant antigen successfully redirected the natural development of detrimental T_H2 cells in infected BALB/c mice.

Why the 24-kD truncated portion of the LACK antigen is so effective in inducing protective immunity remains a matter of speculation but presumably relates to its capacity to target the $CD4^+$ T cells that use the $V_\beta 4$, $V_\alpha 8$ TCR and that are clonally expanded early after natural infection in BALB/c mice (*8*). Although we do not know the biological significance of this phenomenon, our data confirm that there occurs a restricted $V_\beta 4$, $V_\alpha 8$ TCR response to this antigen. BALB/c mice were immunized with p24, and we harvested the lymph node cells and used them to generate T cell hybridomas. Seven consecutive hybridomas that produced IL-2 in response to p24, but not in response to control ovalbumin recombinant protein (that shared the same vector-encoded leader sequence), were analyzed. All used $V_\beta 4$, $V_\alpha 8$ TCR heterodimers (*23*). Although junctional diversity was apparent, the presumptive peptide-binding CDR3 regions of the β chain from all of the hybridomas contained a charged amino acid motif, consisting of Gln-Glu (QE) or Gln-Asp (QD). Similarly, five of six hybridomas established from the lymph node cells of infected BALB/c mice that used the $V_\beta 4$, $V_\alpha 8$ TCR contained the QE motif in the CDR3; one had a charged WD motif at the same position (*8*, *23*). Such findings suggest that the $V_\beta 4$, $V_\alpha 8$ $CD4^+$ T cells expanded during natural infection react to the LACK antigen.

Thus, it is likely that the p24 protein contains an immunodominant epitope that represents the target of the early immune response. The immunodominance of this epitope presumably accounted for its success in protection, in contrast to gp63, an abundant cell surface protease that was not protective under the conditions used. Although the cell biology of *Leishmania* might underlie our ability to identify a dominant MHC class II–associated antigen, it is possible that additional pathogens elicit similar restricted TCR responses early after infec-

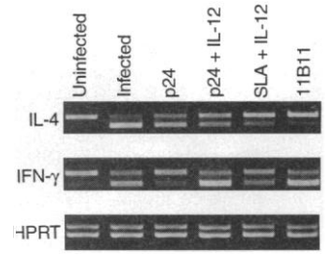

Fig. 5. Immunization with p24 redirected the naturally occurring T_H2 development in infected BALB/c mice. Popliteal lymph nodes draining the site of infection were harvested after 6 weeks from groups of three mice in the designated groups and from uninfected mice and used for the immediate isolation of mRNA. After reverse transcription, cDNA was used in a competitive PCR assay with a polycompetitor construct containing authentic sequences for IL-4, IFN-γ, and the constitutively expressed control gene, hypoxanthine-guanine phosphoribosyltransferase (HPRT), as described (*26*). A constant amount of competitor was used, and the input cDNAs were adjusted until the wild-type amplification product (lower band in each lane on the HPRT gel) from all samples competed equivalently with the larger competitor sequence (upper band in each lane) after HPRT amplification and separation in an ethidium bromide–stained agarose gel. These standardized amounts of cDNA were used in repeated amplifications with primers specific for IL-4 and IFN-γ. This allowed us to quantitate the relative induction of these cytokines among the different samples by their abilities to out-compete the larger competitor construct amplified from the identical primer pairs. We documented band intensities by imaging densitometry to confirm the visual impressions. 11B11 designates mice treated with anti–IL-4.

tion. Analysis of the host T cell response, although indirect, constitutes an approach to reveal the presence of immunodominant antigens expressed early after invasion. This approach would allow vaccine development to concentrate on eliciting strong immune responses during subsequent infection to create the greatest effect in modulating the developing effector cell response.

The pathology, clinical manifestations, and course of leishmanial infections are dependent on a complex interaction between genetically determined virulence characteristics of the *Leishmania* species and the cell-mediated immune responses of the host. In humans, a small subset of people with *L. braziliensis braziliensis* infection develop chronic, mutilating mucosal lesions of the nose, face, or oral pharynx (*24*). Although infected individuals evidence vigorous delayed type hypersensitivity responses to leishmanial antigens, amastigotes are scant in these mucosal lesions, suggesting that an autoimmune response against self-proteins could occur. The 36-kD LACK antigen is highly conserved among related *Leishmania* species and exhibits strong homology with the mammalian RACK1 protein. Although none of the T cell hybridomas against p24 that we have generated react with a murine RACK1 homolog (*14*), it is possible that the high level of conservation between this mammalian protein and its *Leishmania* homolog could explain some of the clinical manifestations of this disease.

We have identified an antigen from *L. major* capable of inducing protection against virulent organisms in susceptible BALB/c mice. The success of this strategy relied on a cloning and screening method for the identification of MHC class II–associated antigens and on the isolation of parasite-specific $CD4^+$ T cell clones that represented a class of cells expanded early after infection. A similar strategy could be used with other infectious diseases in which protective CD4-dependent T cell responses have been described (*25*), thus paving the way for the development of new vaccines.

REFERENCES AND NOTES

1. J.-C. Antoine *et al.*, *Infect. Immun.* **59**, 764 (1991); D. G. Russell, S. Xu, P. Chakraborty, *J. Cell Sci.* **103**, 1193 (1992); T. Lang, R. Hellio, P. M. Kaye, J.-C. Antoine, *ibid.* **107**, 2137 (1994).
2. Z.-E. Wang *et al.*, *J. Immunol.* **151**, 2077 (1993); R. M. Locksley, S. L. Reiner, F. Hatam, D. R. Littman, N. Killeen, *Science* **261**, 1448 (1993).
3. M. Belosevic, D. S. Finbloom, P. H. Van der Meide, M. V. Slayter, C. A. Nacy, *J. Immunol.* **143**, 266 (1989); Z.-E. Wang, S. L. Reiner, D. K. Dalton, R. M. Locksley, *J. Exp. Med.* **179**, 1367 (1994).
4. F. P. Heinzel, M. D. Sadick, S. S. Mutha, R. M. Locksley, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7011 (1991); M. D. Sadick *et al.*, *J. Exp. Med.* **171**, 115 (1990); L. Morris, A. B. Troutt, E. Handman, A. Kelso, *J. Immunol.* **149**, 2715 (1992); F. Y. Liew *et al.*, *Eur. J. Immunol.* **21**, 2489 (1991).
5. P. H. Scott, P. Natovitz, R. L. Coffman, E. Pearce, A. Sher, *J. Exp. Med.* **168**, 1675 (1988); I. Miller, T. Pedrazzini, J. A. Louis, *Immunol. Lett.* **19**, 251 (1988); I. Miller and J. A. Louis, *Eur. J. Immunol.* **19**, 865 (1989); P. H. Scott, E. Pearce, A. W. Cheever, R. L. Coffman, A. Sher, *Immunol. Rev.* **112**, 161 (1989).
6. P. Scott, P. Caspar, A. Sher, *J. Immunol.* **144**, 1075 (1990).
7. I. Miller, J. A. Garcia Sanz, R. Titus, R. Behin, J. A. Louis, *Immunol. Rev.* **112**, 95 (1989).
8. S. L. Reiner, Z.-E. Wang, F. Hatam, P. Scott, R. M. Locksley, *Science* **259**, 1457 (1993).
9. The pET3a-δ9 vector used for the construction of the library is a pET3a-based vector (Novagen, Madison, WI) that was modified by the deletion of the original Eco RI and Hind III restriction sites and by the insertion of the polylinker sequence from the pSP72 vector into the unique Bam HI restriction site. This plasmid was further modified by the addition of two oligonucleotide sequences encoding an HA epitope recognized by mAb 12CA5 and a sequence of six His residues. Both sequences were ligated upstream of the polylinker sequence and in-frame with the 10 amino acids of the leader peptide encoded by pET3a.
10. J. Field *et al.*, *Mol. Cell. Biol.* **8**, 2159 (1988).
11. A vector-primed cDNA library was constructed with *L. major* promastigotes (strain WHOM/IR/−173) as a source of polyadenylated [poly(A)$^+$] RNA. To prepare the vector, we ligated 20 μg of phosphorylated Hind III–poly(T) oligonucleotide primer [AGCT$(T)_{50}$] to 50 μg of Hind III–digested pET3a-δ9. We digested the vector with Sma I to generate a 5′ blunt end and then purified it, first by electrophoresis through a low-melting agarose gel to remove unreacted primers and the short Sma I primer fragment and then by chromatography over oligo(dA) cellulose to purify the poly(T)-tailed vector. Poly(A)$^+$ RNA for the library was isolated through two rounds of oligo(dT) cellulose chromatography from *L. major* promastigotes. To prime cDNA synthesis, we annealed 0.5 μg of tailed vector with 4 μg of poly(A)$^+$ RNA, and first and second strand synthesis was performed with standard methods. The cDNA was blunt-ended with T4 DNA polymerase, and the completed cDNA vector was recircularized. An aliquot of the reaction mixture was electroporated into *E. coli* XL1 Blue and plated on ampicillin plates to determine the titer of the resulting library. For screening, we amplified pools of ~5000 transformed cells in ampicillin-containing semisolid agarose to minimize skewed representation of clones. Bacteria were collected by centrifugation, and aliquots were frozen. The bacteria were grown at 37°C to a density of 3×10^8 cells per milliliter and then infected by the λCE6 bacteriophage (Novagen), which provides the gene for the T7 polymerase. After incubation for 3 hours, cells were harvested by centrifugation, resuspended in 0.5 ml of tris (10 mM, pH 8) per 100 ml of bacteria, and lysed in 9.5 ml of buffer A (6 M guanidinium chloride, 10 mM NaH_2PO_4, and 10 mM tris, pH 8). After continuous stirring for 1 hour, the lysate was centrifugated for 20 min at 4°C, and the clear supernatant sonicated on ice. The bacterial lysate was diluted 10 times in buffer A, and protein purification was performed on affinity columns as indicated by the manufacturer (Qiagen) with buffer E (8 M urea, 10 mM tris, and 100 mM NaH_2PO_4, pH 4.5) for the elution of the recombinant proteins. After purification, the eluates were dialysed extensively first against phosphate-buffered saline (PBS) and then against Dulbecco's minimum essential medium (DMEM).
12. We established bone marrow macrophages by flushing femurs from BALB/c mice and plating the cells at 10^5 cells/ml in Dulbecco's medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 30 mM β-mercaptoethanol, gentamicin (100 μg/ml), and L cell–conditioned media (30% v/v) as a source of granulocyte-macrophage colony-stimulating factor. After 6 days, rat recombinant IFN-γ (100 U/ml) was added to the media, and after 1 day, we harvested the macrophages using cold Ca^{2+}-, Mg^{2+}-free medium to facilitate detachment from the plastic surface.
13. The LMR 16.2 hybridoma was derived from 9.1-2 cells after fusion with BW5147$\alpha^-\beta^-$ lymphoma cells [J. White *et al.*, *J. Immunol.* **143**, 1822 (1989)] by means of polyethylene glycol. T cells hybrids were selected in hypoxanthine, aminopterin, and thymidine medium, cloned by serial dilution, and tested for their ability to secrete IL-2 when incubated with *L. major* SLAs and BALB/c splenocytes. LMR 16.2 was chosen among those that secreted IL-2 when incubated with, but not without SLAs.
14. E. Mougneau, F. Altare, N. Glaichenhaus, unpublished data.
15. The *Leishmania* strains used in this study were *L. major* (strain WHOM/IR/−173), *L. chagasi* (MHOM/BR/74/PP75), *L. donovani* (MHOM/ET/67/HU3: LV9), and *L. amazonensis* (MHOM/BR/73/M2269).
16. D. Ron *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 839 (1994); E. J. Neer, C. J. Schmidt, R. Nambudripad, T. F. Smith, *Nature* **371**, 297 (1994).
17. L. C. C. Afonso *et al.*, *Science* **263**, 235 (1994).
18. V. Colomer-Gould, L. G. Quintao, J. Keithly, N. Nogueira, *J. Exp. Med.* **162**, 902 (1985); L. L. Button and W. R. McMaster, *ibid.* **167**, 724 (1988); *ibid.* **171**, 589 (1990).
19. Z.-E. Wang and R. M. Locksley, unpublished data.
20. S. L. Reiner, S. Zheng, Z.-E. Wang, L. Stowring, R. M. Locksley, *J. Exp. Med.* **179**, 447 (1994).
21. Z.-E. Wang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12932 (1994).
22. ELISPOT assays were performed as follows: Single-cell suspensions were prepared from popliteal lymph nodes and distributed in duplicate aliquots of 10^6 cells to 96-well plates that had been coated with either mAb BVD4-1D11.2 to IL-4 or mAb R46A2 to IFN-γ. Plates were incubated undisturbed for 8 hours at 37°C in a 5% CO_2 atmosphere. After we washed the wells to remove the cells, the wells were incubated with biotinylated secondary antibodies BVD6-24G2.3 or XMG-6 to IL-4 and IFN-γ, respectively, for 1 hour, washed, and incubated further with streptavidin alkaline phosphatase for 1 hour. Color was developed with 5-bromo-4-chloro-3-indolyl phosphate in 0.1 M 2-amino-2-methyl-1-propanol buffer suspended in agarose, and after solidification of the agar, individual blue spots were counted with inverted microscopy.
23. Total mRNA was isolated from individual T cell hybridomas with RNAzol (Biotecx, Houston, TX). We used Moloney murine leukemia virus reverse transcriptase (Gibco-BRL), deoxynucleotide triphosphates, and an antisense primer from the constant regions of either the α or β chain of the TCR to reverse transcribe sequences of interest. Amplification with PCR was done with a set of degenerate oligomers from the 5′ variable regions of the α or β TCR together with nested 3′ oligomers from the C_α or C_β regions. Primer sequences were as described (*8*). The PCR products were isolated from agarose gels and sequenced directly with the dideoxy chain termination method with the Sequenase kit (U.S. Biochemical).
24. T. C. Jones *et al.*, *J. Infect. Dis.* **156**, 73 (1987).
25. D. A. Brake, W. P. Weidanz, C. A. Long, *J. Immunol.* **137**, 347 (1986); *ibid.* **140**, 1939 (1988); S. P. Nickell, A. Gebremichael, R. Hoff, M. Boyer, *ibid.* **138**, 914 (1987); A. W. Taylor-Robinson, R. S. Phillips, A. Severn, S. Moncada, F. Y. Liew, *Science* **260**, 1931 (1993).
26. S. L. Reiner, S. Zheng, D. B. Corry, R. M. Locksley, *J. Immunol. Methods* **165**, 37 (1993).

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