10. S. Cotecchia *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7159 (1988); C. J. Lynch *et al.*, *Mol. Pharmacol.* **29**, 196 (1986).
11. D. Wu, A. Katz, C.-H. Lee, M. I. Simon, *J. Biol. Chem.*, in press; S. Cotecchia, unpublished data.
12. D. A. Schwinn *et al.*, *J. Biol. Chem.* **265**, 8183 (1990).
13. D. A. Schwinn *et al.*, *Mol. Pharmacol.* **40**, 619 (1991).
14. G. Berstein *et al.*, *J. Biol. Chem.* **267**, 8081 (1992).
15. E. G. Peralta *et al.*, *EMBO J.* **6**, 3923 (1987).
16. 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; X, a basic amino acid; and Y, Tyr.
17. J. Kebabian and D. Caine, *Nature* **277**, 93 (1979).
18. B. R. Conklin *et al.*, *J. Biol. Chem.* **267**, 31 (1992).
19. A. H. Cheung, C. H. Ruey-Ruey, M. P. Graziano, C. D. Strader, *FEBS Lett.* **279**, 277 (1991); T. Okamoto *et al.*, *Cell* **67**, 723 (1991).
20. T. Okamoto and I. Nishimoto, *J. Biol. Chem.* **267**, 8342 (1992).
21. H. M. Dalman and R. R. Neubig, *ibid.* **266**, 11025 (1991).
22. B. König *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6878 (1989).
23. H. Mukai, E. Munekata, T. Higashijima, *J. Biol. Chem.* **267**, 16237 (1992).
24. We used the polymerase chain reaction (PCR) to construct a set of minigenes containing fragments derived from the 3i loop of the α_{1B}-AR or D_{1A}-dopamine receptor spliced with the 3′-untranslated region (3′-UTR) of the human β-globin gene. We added the β-globin gene 3′-UTR to enhance the stability of mRNA. Each minigene contained a restriction site at the 5′ end for subcloning, followed by the ribosome-binding site consensus sequence GCCGCCACCATGG [M. Kozak, *J. Cell Biol.* **108**, 229 (1989)]. In all constructs, a methionine codon was added upstream from the receptor-specific sequences as a translation initiation site and was followed by a glycine codon (GGA). We chose glycine as the second amino acid to protect the consensus sequence for ribosome binding during translation and to protect the nascent polypeptide against proteolytic degradation. At the end of the α_{1B}-3i, α_{1B}-3iN, or α_{1B}-3iC coding segment, a TAA stop codon was added, followed by a restriction site and the β-globin gene 3′-UTR. At the 3′ end of each construct, restriction sites were engineered for subcloning. The D_{1A} 3i minigene construct was similarly prepared. All restriction sites and signaling sequences and the splice site between the 3i loop coding region and the β-globin gene 3′-UTR were introduced by synthetic oligonucleotides during PCR amplification. Oligonucleotides were synthesized on a DNA synthesizer (model 380A; Applied Biosystems). Amplification of DNA fragments was performed as described [R. M. Horton, R. D. Hunt, S. N. Ho, J. K. Pullen, L. R. Pease, *Gene* **77**, 61 (1989)]. Splicing reactions at strictly defined points between the peptide coding regions and the β-globin gene 3′-UTR were done with a modification of the general polymerase-mediated recombination protocol [R. K. Saika *et al.*, *Science* **239**, 487 (1988)], as described (*4*). After PCR amplification, the full-length minigene DNA fragments were cloned by standard methods into the eukaryotic expression vector pRK5.
25. J. R. Didsbury *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 11564 (1991).
26. H. Schägger and G. von Jagow, *Anal. Biochem.* **166**, 368 (1987).
27. D. B. Smith and K. S. Johnson, *Gene* **67**, 31 (1988).
28. Y. Salomon, *Methods Enzymol.* **195**, 22 (1991).
29. We thank E. G. Peralta for providing the cDNA for the human M1 AChR; T. Kurose for providing the α_{1B}-3i loop glutathione S-transferase fusion protein; Q. Ren for assistance in preparing DNA constructs; and M. G. Caron for providing the cDNA for the D_{1A} dopamine receptor, for discussions, and for critical reading of this manuscript. Supported in part by NIH grant HL16037.

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T_H1 and T_H2 Cell Antigen Receptors in Experimental Leishmaniasis

Steven L. Reiner, Zhi-En Wang, Farah Hatam, Phillip Scott, Richard M. Locksley*

The complexity and chronicity of parasitic infections have obscured the identification of biologically relevant antigens. Analysis of the T cell receptor repertoire used by mice infected with *Leishmania major* revealed the expansion of a restricted population of CD4$^+$ cells. These cells expressed the V_α8-J_αTA72,V_β4 heterodimer in both progressive infection and protective immunity and across several major histocompatibility haplotypes. Thus, the same immunodominant parasite epitope drives the disparate outcomes of this infectious process, suggesting that candidate vaccine antigens selected by screening of immune individuals may be capable of exacerbating disease in genetically susceptible individuals.

A number of infections, including leprosy, leishmaniasis, and tuberculosis, have polarized manifestations that range from asymptomatic seroconversion to disseminated disease. Infection of BALB/c mice with *L. major* leads to parasitization of macrophages and progressive disease that closely mimics human visceral leishmaniasis, or kala-azar. Disease has been associated with expansion of the T helper 2 (T_H2) CD4$^+$ population. These cells contain transcripts for interleukin-4 (IL-4) and IL-10 (*1*), cytokines that interfere with the activation of infected macrophages to a leishmanicidal state (*2*). Several immunologic manipulations, including transient CD4$^+$ depletion (*3*) or administration of antibody to IL-4 (*4*), permit these animals to establish a healer phenotype comparable to genetically resistant mouse strains such as C57BL/6 and C3H/HeN. This phenotype has been associated with expansion of T_H1 CD4$^+$ cells that contain transcripts for interferon-γ (IFN-γ) (*1*, *4*). Transferred CD4$^+$ cell lines of the T_H1 or T_H2 type are capable of establishing a healer or nonhealer phenotype, respectively, in a manner dependent on the elaboration of one of two key cytokines, IFN-γ or IL-4 (*5*). Administration of neutralizing antibodies to IFN-γ or IL-4 can reverse a healer or nonhealer phenotype, respectively, but such interventions must occur close to the time of infection (*4*, *6*). Although leishmaniasis, like most parasitic infections, is a chronic disease, these experiments implicate early events in determining the critical CD4$^+$ T cell response that mediates the eventual outcome. To define the relation between parasite antigens capable of eliciting protective or nonprotective immune responses in a characterized model, we analyzed T cell receptor (TCR) usage at early time points.

The expansion of CD4$^+$ T cells in BALB/c mice (nonhealer phenotype), BALB/c mice induced to heal by treatment with antibody to IL-4 (anti–IL-4), and in C57BL/6 mice (healer phenotype) was characterized by monoclonal antibodies (MAbs) to most of the variable regions of TCRβ molecules. MAbs to four TCRα chains were also analyzed (Fig. 1). As measured by flow cytofluorometric analysis, few differences were evident between animals that cleared the infection and those that developed disease or between different major histocompatibility (MHC) haplotypes (BALB/c mice are *H-2^d* and C57BL/6 mice are *H-2^b*). V_β4-bearing CD4$^+$ cells were expanded three to four times in all experimental groups. Among the V_α families analyzed, V_α8 was also increased four to seven times by 2 weeks after inoculation of the parasite.

To assess TCR usage among parasite-specific T helper cells, we established 16 consecutive *Leishmania*-specific T_H2 clones (*7*) from six animals with progressive infection and cloned and sequenced the TCRs (Table 1). A strong bias toward V_α8 (50% of clones) and V_β4 (31%) usage was apparent: in three of the clones these gene products were paired and more than half of the $V_\alpha8^+$ T_H2 clones paired with J_αTA72 and had identical junctional nucleotide sequences. The pairings of V_β4 with J_β gene products were more diverse.

Three *L. major*–specific T_H1 clones were established from mice induced to heal after treatment with anti-CD4. An additional T_H1 clone, 9.1-2, which was established from a BALB/c mouse immunized by intravenous injection of parasite antigens, confers protection after adoptive transfer (*8*). Three of these four clones, including 9.1-2, contained the identical V_α8-J_αTA72 sequence found in several of the T_H2 clones. Two of these three clones used the V_β4-

S. L. Reiner, Z-E. Wang, F. Hatam, R. M. Locksley, Department of Medicine and Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA 94143.
P. Scott, Department of Pathobiology, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104.
*To whom correspondence should be addressed.

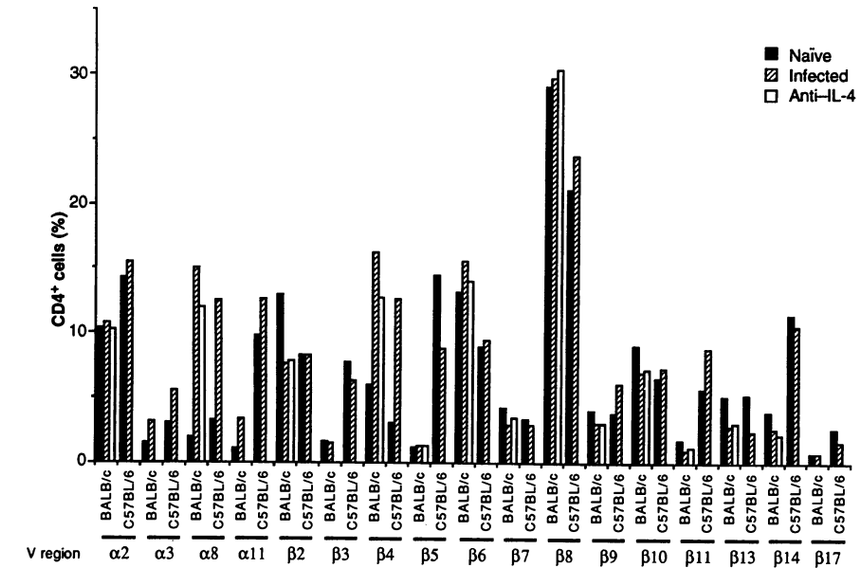

Fig. 1. Analysis of T cell receptor usage by CD4+ cells during leishmaniasis. Popliteal lymph node cells (LNCs) were harvested from naïve mice and animals infected with *L. major* as described (*1*), teased into single-cell suspensions at 4°C, washed, and incubated with the designated MAbs or propidium iodide before analysis to determine the logarithm of fluorescence emission with a fluorescence-activated cell sorter (FACScan; Becton Dickinson). Macrophages and neutrophils were excluded by forward- and side-scatter analysis. Phycoerythrin (PE)-conjugated antibody to CD4 (MAb GK1.5; Becton Dickinson) was used in conjunction with the appropriate antibody to V region that was conjugated with either fluorescein isothiocyanate (FITC) or biotin. Streptavidin-tricolor (CalTag) was used as a second-step label for biotinylated antibodies. Values given are the results of a typical experiment or the mean of many experiments.

The expansion of $V_\alpha 8,V_\beta 4$ CD4+ cells was analyzed in vivo by flow cytometry of lymphocytes harvested at designated times after inoculation of the parasite (Fig. 2). These studies demonstrated that the peak expansion of $V_\alpha 8,V_\beta 4$ CD4+ cells (9 to 75 times the baseline numbers in various experiments) occurred 2 weeks after infection, before the disease becomes clinically manifest. By the sixth week, CD4+ cells expressing $V_\alpha 8$, $V_\beta 4$, or both, continued to be present in greater frequency than in naïve controls.

As independent confirmation of the flow cytometry studies, quantitative polymerase chain reaction (PCR) was used to assess the expression of $V_\alpha 8$ and $V_\beta 4$ mRNA among CD4+ cells taken from draining lymph nodes during infection and from uninfected mice (Fig. 3A). $V_\alpha 8$ and $V_\beta 4$ mRNAs were expanded four- to sixfold in both healing and progressive infection when normalized to mRNA amplified from the constant region of the other chain of the heterodimeric TCR. The ability of this assay to detect expansion of discrete subpopulations of CD4+ cells was confirmed with the use of a $V_\beta 13$-specific oligomer that documented no significant expansion of these cells (Fig. 3A). A similar analysis was used to examine levels of IL-4 and IFN-γ mRNA in $V_\alpha 8$-expressing T cells isolated 2 weeks after infection. Polarized lymphokine mRNA transcripts for either IFN-γ or IL-4 were already present in the $V_\alpha 8^+$ populations (Fig. 3B), demonstrating that a T cell subset with a relatively limited receptor

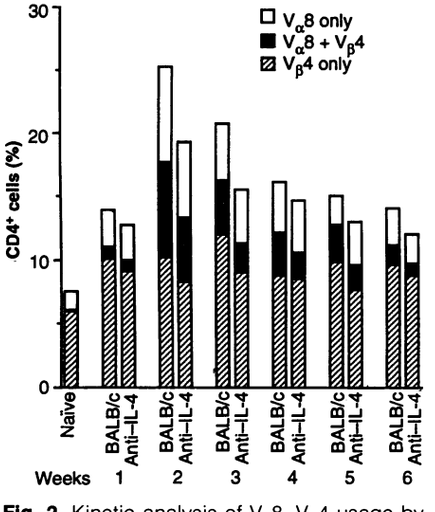

Fig. 2. Kinetic analysis of $V_\alpha 8$, $V_\beta 4$ usage by CD4+ cells during leishmaniasis in BALB/c mice. LNCs were isolated and stained as described in Fig. 1. FITC-conjugated MAb KT 50 [anti-$V_\alpha 8$; (*21*)] and biotinylated MAb KT4 [anti-$V_\beta 4$; (*22*)] secondarily labeled with streptavidin-tricolor (CalTag) were used to assess staining among CD4+ lymphocytes by selecting for analysis only cells staining with PE-conjugated MAb GK1.5. Gates for $V_\alpha 8$ and $V_\beta 4$ were determined by the staining pattern of single and double antibody–stained controls plus parallel specimens stained with propidium to assess viability.

$J_\beta 1.6$ sequence found in the $T_H 2$ clone U3; the third used $V_\beta 4$ but was paired to a different J_β. Expression was confirmed by staining with MAbs to $V_\alpha 8$ and $V_\beta 4$.

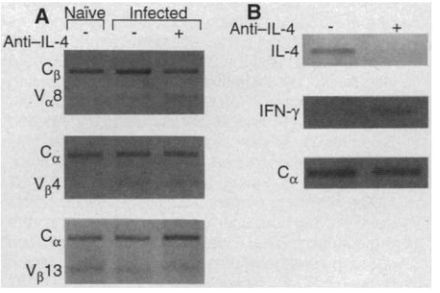

Fig. 3. Quantitative PCR assay of mRNA isolated from the LNCs of uninfected, infected, or anti-IL-4–pretreated and infected BALB/c mice collected at 2 weeks after infection with *L. major*. (**A**) TCR expression before and during leishmaniasis among CD4-selected cells. (**B**) Cytokine profile of $V_\alpha 8$-bearing T cells during progressive and resolving infection. We isolated CD4+ LNCs to >93% purity using magnetic bead selection with MAb GK1.5 as described (*1*) from uninfected or infected BALB/c mice (A). We purified $V_\alpha 8^+$ cells from parallel specimens using magnetic bead selection with MAb KT-50 (B). Total RNA was extracted with RNAzol (Biotecx) and reverse-transcribed with random hexamer primers for use as PCR template. Equal amounts of RNA (as measured by both optical density and ethidium bromide staining in agarose gels) were used in each reaction. Thermocycle conditions were established in preliminary experiments to reflect direct proportionality between input RNA and intensity of the amplified product. TCR variable region quantitation was internally controlled by simultaneous amplification of the constant region from the heterodimeric partner chain as described (*23*). Amplification of IL-4 and IFN-γ was standardized by concurrent amplification of TCR C_α. Primers used (in addition to C region primers listed in legend to Table 1) were as follows: $V_\alpha 8$ (5′-TGAAGAGCTCCACAGACAACAAG-3′), $V_\beta 4$ (5′-AGTCGCTTCCAACCTCAAAG-3′), $V_\beta 13$ (5′-CCCTCGGATCGATTTTCTGCT-3′), C_α sense (5′-CCCAGAACCTGCTGTGTACCA-3′), C_β sense (5′-TGTGACTCCACCCAAGGT-3′), IL-4 (antisense, 5′-CTTTAGGCTTTCCAGGAAGTCTTT-3′; sense, 5′-CTAGTTGTCATCCTGCTCTTCTTT-3′), and IFN-γ (antisense, 5′-GTGGACCACTCGGATGAGCTCATT-3′; sense, 5′-TGTTACTGCCACGGCACAGTCATT-3′). PCR products were separated on a 2% agarose gel, stained with ethidium bromide, and photographed with positive-negative film (Polaroid type 55) to allow quantitation of band intensity by video densitometry (Joyce-Loebl) of the negative image. The authenticity of the amplified products was confirmed by direct sequencing or Southern (DNA) blotting and hybridization, with an internal oligomer, or both.

repertoire can manifest opposite functional phenotypes in vivo.

Mouse strains of different MHC class II haplotypes that are genetically susceptible (BALB/b; *H-2*^{b}) or resistant (B10.D2, *H-2*^{d}; C57BL/6, *H-2*^{b}; C3H/HeN, *H-2*^{k}) to *L. major* were compared for expression of $V_\alpha 8$ and $V_\beta 4$ among $CD4^+$ cells during infection (Fig. 4). In BALB/c (nonhealer) and B10.D2 (healer) mice (*H-2*^{d}) there was expansion of cells expressing one or both of the markers. The number of $V_\alpha 8^+$ cells paired with $V_\beta 4^+$ cells was expanded as compared with the naïve state. BALB/b (nonhealer) and C57BL/6 (healer) mice (*H-2*^{b}) also showed marked expansion of cells expressing both $V_\alpha 8$ and $V_\beta 4$. Mice with the *H-2*^{b} haplotype displayed an increased number of $V_\beta 4^+$ cells, although the numbers of $V_\alpha 8^+$ and $V_\alpha 8^+V_\beta 4^+$ cells were as greatly expanded as in *H-2*^{d} animals. C3H/HeN (healer) mice (*H-2*^{k}) had similar expansion of the $V_\alpha 8^+$ cells but less bias in the pairing with $V_\beta 4$ and little expansion of the $V_\beta 4^+$ population. Triple staining with MAbs to $V_\alpha 2$, $V_\beta 14$, and CD4 showed that the presence of multiple stains did not falsely elevate the values of TCR expression during infection.

We evaluated the prototypic TCRα gene rearrangement occurring in clone 9.1-2 in infected mice of the different MHC class II haplotypes to determine whether the conserved heterodimeric TCR shared clonal specificity. PCR amplification with $V_\alpha 8$- and J_αTA72-specific oligomers showed increased amounts of this TCRα chain in genomic DNA extracted from infected lymph nodes as compared with spleen cells from uninfected animals, as assessed by probing the amplification product with an internal $V_\alpha 8$ oligomer (Fig. 5). We assessed the fine specificity by probing the blot with an oligomer specific for the junctional sequence of the prototypic clones and normalizing the hybridization signal to that of the total $V_\alpha 8$-J_αTA72 product. Infected BALB/c of both healer and nonhealer phenotypes contained the highest proportion of the 9.1-2–like rearrangement, although hybridization occurred in all *L. major*–infected mice.

These findings provide insight into the development of the T_H1 and T_H2 responses

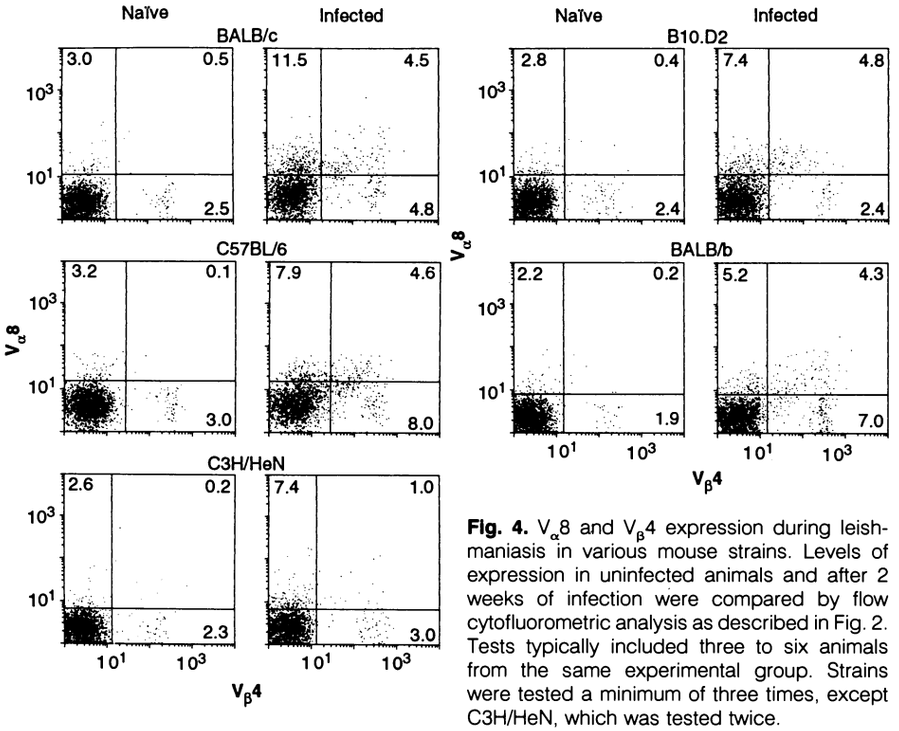

Fig. 4. $V_\alpha 8$ and $V_\beta 4$ expression during leishmaniasis in various mouse strains. Levels of expression in uninfected animals and after 2 weeks of infection were compared by flow cytofluorometric analysis as described in Fig. 2. Tests typically included three to six animals from the same experimental group. Strains were tested a minimum of three times, except C3H/HeN, which was tested twice.

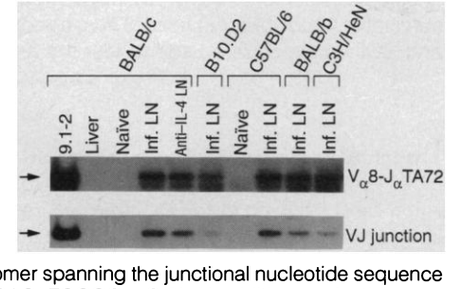

Fig. 5. Expansion of cells containing a $V_\alpha 8$-J_αTA72 rearrangement in various mouse strains during leishmaniasis. Genomic DNA was isolated from the various experimental groups and digested with Eco RI. Amplification was carried out on 10 ng of DNA with the $V_\alpha 8$ 5′ primer (Fig. 3) and an antisense primer from the intron 3′ of J_αTA72 (5′-TTCAGAATTCCTACTCTACATAGGA-3′) for 35 cycles. PCR products were resolved on a 2% agarose gel and transferred to a nylon membrane (Hybond). Hybridization under high stringency conditions was done with an oligomer spanning the junctional nucleotide sequence found in clone 9.1-2 (5′-TGTGCTTTGAGTGCCGACATGGGCTAC-3′; N addition nucleotides are underlined; the 13 bases on the 5′ end are derived from $V_\alpha 8$, and the 11 bases on the 3′ end are derived from J_αTA72). Blots were then stripped and reprobed with an internal oligomer for $V_\alpha 8$ (5′-AGCACAGAAGTACAGGGCA-3′) to ascertain the amounts of the total PCR product.

Table 1. V and J region distribution among *Leishmania*-specific $CD4^+$ clones. Total mRNA was isolated from the clones or hybridomas with RNAzol (Biotecx). Moloney murine leukemia virus reverse transcriptase (BRL), deoxynucleotide triphosphates, and an antisense primer from the constant (C) region of either the α (5′-TCAACTGGACCACAGCCTCAG-3′) or β (5′-GATCTCATAGAGGATGGT-3′) chains of the TCR were used to reverse transcribe the mRNA of interest. Amplification with PCR was done with a set of degenerate oligomers from the 5′ variable regions of the α [5′-A(G,T)TAC-(A,T)TCT(A,T)(C,T)TGGTAC-3′ and 5′-C(A,T)(A,G,T)(A,C)(C,T)CT(G,T)TTCTGGTA(C,T)-3′] or β [5′-ATGTACTGGTA(C,T)(A,C)(A,A,G)(A,G)-CA-3′] TCR together with nested 3′ oligomers from the C_α (5′-GATCTTTTAACTGGTACACAG-3′) or C_β (5′-GGAGACCTTGGGTGGAGTCAC-3′) regions. Anchored PCR as described in (*24*) was required to amplify the productively rearranged gene product in some instances. The PCR products were isolated from agarose gels and sequenced directly with the dideoxy chain termination method with the use of the Sequenase kit (U.S. Biochemical Corp.). Assignment of gene usage was based on nomenclature for sequences listed in GenBank. $V_\alpha 8$ sequences were homologous with BALB/c cosmid $V_\alpha 8.2A$ (provided by K. Wang and L. Hood) and are equivalent to designation $V_\alpha F3.4$ of Chou *et al.* (*25*).

Clone	V_α-J_α	V_β-J_β
	T_H2	
U3	8-TA72	4 –1.6
U2	8-TA72	6 –2.7
U4	8-DK	4 –2.7
1.2	8-LB2	8.3–1.4
2.2	8-TA72	8.2–2.2
2.3	2-LB2	15 –1.4
3.5	4-TA28	13 –2.4
4.1	5-TA72	6 –1.6
4.3	8-TA72	9 –2.7
4.4	5-TA31	2 –2.3
5.3	14-E1	4 –2.2
M1	4-C5	15 –2.3
M2	3-TT11	4 –2.5
M3	3-TA13	15 –1.5
M5	8-N14	8.3–2.7
M6	8-TA72	4 –2.7
	T_H1	
H27	8-TA72	4 –1.6
113	8-TA72	4 –1.6
4.10	8-TA72	4 –2.3
9.1-2	8-TA72	4 –1.6

that have been implicated in many bacterial, mycobacterial, fungal, and parasitic diseases (*9*). There are two models for the development of T_H1 and T_H2 responses: the first suggests that they derive from distinct precursors (*10*), whereas the second suggests that they arise from a common lineage. The use of peptide-specific clones (*11*), limiting dilution analysis (*12*), and transgenic mice expressing a single TCR (*13*) support the second possibility, and the findings in this paper extend these previous observations by linking subset maturation to biologically important antigens implicated in the immune response to an infectious organism.

Evidence supporting an oligoclonal immune response was obtained by flow cytofluorometric analysis, direct sequencing of receptors from parasite-specific $CD4^+$ T cell clones, and amplification of genomic rearrangements as extracted from infected mice. The response had unusual features. First, several of the clones used common variable region genes but expressed distinct junctional sequences. The junctional diversity created by N region additions and exonucleotytic nibbling generates the CDR3 domain of the TCR implicated in the interaction with peptide (*14*). Although this implies that these different clones should recognize distinct epitopes, instances in which conserved V regions seem to contribute substantially to fine specificity despite CDR3 differences have been noted (*15*). Second, 90% of the deduced junctional sequences of the *Leishmania*-specific clones contain anionic residues in this region (*16*), perhaps indicating recognition of a cationic parasite antigen (*17*). The importance of charged residues within the CDR3 domain in shaping the TCR-peptide interaction with MHC has been delineated with the use of transgenic mice expressing single-chain TCRs (*18*). Third, the occurrence of relatively conserved TCR sequences among different animals and across MHC class II boundaries was not predicted. The generation of the rearranged functional TCR is not random; bias in the use of junctional nucleotide additions contributes to constraints on CDR3 length (*19*). The frequent use of the $V_\alpha8$-J_αTA72 rearrangement suggests that the CDR3 region of the TCRα chain may interact with a monomorphic parasite determinant, whereas the variation in pairing with $V_\beta4$ or in the $V_\beta4$-D-J_β recombination may provide the necessary polymorphism to bind different MHC molecules.

The nature of the antigen constitutes an important area for further investigation. Experiments to date have identified reactivity in association with a 10-kD protein that stimulates the protective BALB/c-derived clone, 9.1-2 (*8*). The identification of this antigen in all *Leishmania* species analyzed (*8*) is consistent with its important role in the immune response and in disease. As shown in the present study, however, the immunologic milieu, rather than the antigen, may be more important in determining the development of a successful immune response. A search for factors that modify the developmental regulation of $CD4^+$ subsets might ultimately be more productive than the hope that universally protective microbial antigens can be reliably identified.

REFERENCES AND NOTES

1. F. P. Heinzel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7011 (1991); F. P. Heinzel *et al.*, *J. Exp. Med.* **169**, 59 (1989).
2. M. Lehn *et al.*, *J. Immunol.* **143**, 3020 (1989); C. Bogdan *et al.*, *J. Exp. Med.* **174**, 1549 (1991); R. de Waal Malefyt *et al.*, *ibid.*, p. 915.
3. R. G. Titus, R. Ceredig, J.-C. Cerottini, J. A. Louis, *J. Immunol.* **135**, 2108 (1985).
4. M. D. Sadick *et al.*, *J. Exp. Med.* **171**, 115 (1990).
5. B. J. Holaday *et al.*, *J. Immunol.* **147**, 1653 (1991); P. Scott, P. Natovitz, R. L. Coffman, E. Pearce, A. Sher, *J. Exp. Med.* **168**, 1675 (1988).
6. M. Belosevic *et al.*, *J. Immunol.* **143**, 266 (1989); R. Chatelain, K. Varkila, R. L. Coffman, *ibid.* **148**, 1182 (1992).
7. We selected clones 9.1-2 and H27 and U2, U3, and U4 by limiting dilution from characterized *Leishmania*-specific T_H1 and T_H2 $CD4^+$ cell lines that adoptively transfer protection or exacerbation, respectively (*5*). Clone 9.1-2 is a protective $CD4^+$ clone derived from an immunized BALB/c mouse (*8*). The remaining TCR sequences were generated from hybridomas established by fusion of $CD4^+$ lymph node cells from healing (that is, previously treated with anti-CD4) BALB/c mice or BALB/c mice with progressive infection with the TCRα- and TCRβ-negative mutant thymoma cell line, BW 5147 1100.129.237 (*20*). Viable clones were selected for analysis only if they expressed CD4 and TCR, as assessed by fluorescence analysis, and released either IFN-γ or IL-4 in response to stimulation with a sonicate of *Leishmania* antigen plus antigen-presenting cells. The cytokine response was only transiently expressed in these hybridomas but permitted the assignment of one-third of the viable hybridomas to a T_H1 or T_H2 phenotype.
8. P. Scott, P. Caspar, A. Sher, *J. Immunol.* **144**, 1075 (1990).
9. G. Del Prete *et al.*, *J. Clin. Invest.* **88**, 346 (1991); H. Yssel *et al.*, *J. Exp. Med.* **174**, 593 (1991); J. B. A. G. Haanen *et al.*, *ibid.*, p. 583; M. Yamamura *et al.*, *Science* **254**, 277 (1991); P. Salgame *et al.*, *ibid.*, p. 279; L. Romani *et al.*, *J. Exp. Med.* **176**, 19 (1992); A. Sher and R. L. Coffman, *Annu. Rev. Immunol.* **10**, 385 (1992).
10. N. E. Street and T. R. Mosmann, *FASEB J.* **5**, 171 (1991).
11. B. D. Evavold *et al.*, *J. Immunol.* **148**, 347 (1992).
12. M. Rocken, J.-H. Saurat, C. Hauser, *ibid.*, p. 1031.
13. R. A. Seder, W. E. Paul, M. M. Davis, B. F. de St. Groth, *J. Exp. Med.* **176**, 1091 (1992).
14. J. L. Jorgensen, P. A. Reay, E. W. Ehrich, M. M. Davis, *Annu. Rev. Immunol.* **10**, 835 (1992).
15. B. Boitel *et al.*, *J. Exp. Med.* **175**, 765 (1992); J.-L. Casanova, P. Romero, C. Widmann, P. Kourilsky, J. L. Maryanski, *ibid.* **174**, 1371 (1991).
16. S. L. Reiner, unpublished data.
17. S. Adams, P. LaBlanc, S. K. Datta, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 11271 (1991).
18. J. L. Jorgensen *et al.*, *Nature* **355**, 224 (1992).
19. S. Candeias, C. Waltzinger, C. Benoist, D. Mathis, *J. Exp. Med.* **174**, 989 (1991).
20. J. White *et al.*, *J. Immunol.* **143**, 1822 (1989).
21. K. Tomonari, E. Lovering, S. Fairchild, S. Spencer, *Eur. J. Immunol.* **19**, 1131 (1989).
22. K. Tomonari, E. Lovering, S. Spencer, *Immunogenetics* **31**, 333 (1990).
23. Y. Choi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8941 (1989).
24. A. H. Taylor, A. M. Haberman, W. Gerhard, A. J. Caton, *J. Exp. Med.* **172**, 1643 (1990).
25. H. S. Chou *et al.*, *EMBO J.* **5**, 2149 (1986).

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Inhibition of Human Colon Cancer Growth by Antibody-Directed Human LAK Cells in SCID Mice

Hiroshi Takahashi,* Tetsuya Nakada, Isabelle Puisieux

Advanced human colon cancer does not respond to lymphokine-activated killer (LAK) cells. In order to direct cytotoxic cells to the tumor, human LAK cells linked with antibodies to a tumor cell surface antigen were tested with established hepatic metastases in severe combined immunodeficient (SCID) mice. These cells had increased uptake into the tumor and suppression of tumor growth as compared with LAK cells alone, thereby improving the survival of tumor-bearing mice. Thus, tumor growth can be inhibited by targeted LAK cells, and SCID mice can be used to test the antitumor properties of human effector cells.

Lymphokine-activated killer cells generated by interleukin-2 (IL-2) are cytotoxic to colon cancer cells in vitro (*1*). However, in clinical trials, advanced human colon cancer has been refractory to adoptive immunotherapy using LAK cells (*2*). This limitation of LAK therapy may be due in part to an ineffectiveness of LAK cell cytotoxicity (*3*) or a lack of recruitment of LAK cells to the tumor mass (*4*). In this report, we demonstrate that human LAK cells can be targeted to the tumor by antibodies to a tumor cell surface antigen. This targeting augments the LAK cell effectiveness against hepatic metastases of human colon cancer in SCID mice (*5*).

SCID mice have no functional T and B cells and therefore will not reject xenografts