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39. The DNA model proved straightforward to construct by making simple rotations and translations of the helical axes that enabled the 5′ to 3′ and 3′ to 5′ phosphate backbone strands of each duplex section to be joined to those of adjacent sections. The covalent bond distances and angles are those of standard B-DNA (*55*), except for the ε and ζ torsion angles adopted by the single linking phosphate group at the 90° bend in each chain, which have values of 276° and 222°, respectively. These torsion angles are stereochemically reasonable and compare with values for ε of 170° and for ζ of 257° in straight standard B-DNA (*55*).
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Resistance to *Leishmania major* Induced by Tolerance to a Single Antigen

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In mice, susceptibility to *Leishmania major* is associated with the early expansion of T helper 2 cells (T_H2) cells, but nothing is known of the specificity of these cells. A previously identified antigen, *Leishmania* homolog of receptors for activated C kinase (LACK), was found to be the focus of this initial response. Mice made tolerant to LACK by the transgenic expression of the antigen in the thymus exhibited both a diminished T_H2 response and a healing phenotype. Thus, T cells that are activated early and are reactive to a single antigen play a pivotal role in directing the immune response to the entire parasite.

The outcome of an infection can be determined by the balance between interferon-γ (IFN-γ)–secreting (T_H1) and interleukin-4 (IL-4)–secreting (T_H2) cells (*1*). In experimental murine leishmaniasis, susceptible mice such as those of the BALB/c strain respond to infection with the preferential expansion of IL-4–producing T_H2 cells (*2*). Neutralization of IL-4 within the first week of infection prevents the emergence of the T_H2 response and allows the generation of potentially protective T_H1 cells and the development of a healing phenotype (*3*). IL-4 appears to be both the main inducer of T_H2 responses and an inhibitor of T_H1 responses (*4*). An early burst of IL-4 production is detected in the lymph nodes of infected mice, with $CD4^+$ T cells being the main cellular source (*5*). It has been proposed that these early-activated IL-4–secreting cells belong to the recently described subpopulation of natural killer (NK) T cells that react to the nonclassical major histocompatibility complex (MHC) class I protein CD1 (*6*), but recent experiments using β_2-microglobulin–deficient mice, in which NK T cells are not positively selected, do not support this hypothesis (*7*). Alternatively, these IL-4–secreting $CD4^+$ T cells may be parasite-specific cells that are activated very rapidly after infection. The work described here is aimed at identifying the parasite antigens that trigger this early burst of IL-4 and at down-regulating this response by means of antigen-specific tolerogenic approaches.

We recently described the expression cloning of a 36-kD *Leishmania* antigen, LACK (*8*). Most LACK-reactive T cells used the same $V_\alpha8$ and $V_\beta4$ variable T cell receptor regions and reacted to the same antigenic determinant (amino acids 158 through 173). Expansion of $V_\alpha8^+V_\beta4^+$ $CD4^+$ T cells occurred in the lymph nodes of infected mice (*9*), which suggests that LACK was the focus of the initial immune response against the parasite (*8*). To test this, we generated a panel of 30 parasite-specific short-term T cell clones from the lymph nodes of infected BALB/c mice (*10*). Ten of these clones responded to LACK; all secreted IL-4 but not IFN-γ, which suggests that LACK was a preferential target of the early anti-parasite immune response and that early-activated LACK-reactive T cells exhibited a T_H2 phenotype. This was confirmed by monitoring of the number and the phenotype of LACK-reactive T cells by means of an antigen-specific ELISPOT assay (Fig. 1). Six days after infection, lymph node $CD4^+$ T cells were prepared and incubated with syngeneic antigen-presenting cells (APCs) with or without an optimal concentration of LACK peptide or soluble extracts from the parasite soluble *Leishmania* antigens (SLA)] (*11*). Infection induced a strong response against LACK, and most of the early-activated T cells secreted IL-4 or IL-5 or both (Fig. 1). This contrasts with the exclusively T_H1 response directed to the main antigenic determinant of the major promastigote surface protease, GP63 (*12*), whereas responses to other parasite determinants, including the recently identified LDP23 antigen (*13*), were of lesser magnitude.

The early burst of LACK-induced T_H2-

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like cytokines in BALB/c mice contributes to the initial cytokine milieu in a way that may subsequently favor the differentiation of other T_H2 cells (*4*). If this is correct, susceptible mice manipulated to be tolerant to LACK might switch from a detrimental T_H2-biased to a protective T_H1-biased response. To test this, we generated transgenic mice that carried a truncated LACK cDNA driven by the MHC class II I-Eα promoter (*14*). These transgenic animals, designated IE-LACK, were backcrossed to BALB/c mice for eight generations. LACK transcripts were detected in primary and secondary lymphoid organs, including the thymus, spleen, and bone marrow, but not in the liver, kidneys, and muscles. To demonstrate tolerance, IE-LACK transgenic mice were immunized with LACK in incomplete Freund's adjuvant (IFA). Ten days after immunization, lymph node $CD4^+$ T cells were prepared and cultivated with LACK. T cells from control littermates proliferated strongly and secreted large amounts of IL-4, IL-5, and IFN-γ when incubated with LACK, but no proliferation or cytokine secretion was observed with T cells from transgenic mice (Fig. 2) (*15*). Thus, both T_H1 and T_H2 responses were tolerized in IE-LACK transgenic mice. These mice did mount an immune response against a nonrelated control antigen.

IE-LACK transgenic mice infected with *L. major* were still alive and well 6 months after inoculation with the parasite, whereas all control littermates developed progressive lesions and died within 20 weeks. Differences between the two groups of mice could be detected as early as 6 days after infection (Fig. 3). LACK-reactive cells were detected only in control animals and were of T_H2 phenotype. Control mice contained eight times as many T cells that secreted IL-4 in response to SLA as did transgenic littermates. The nature and the number of GP63-reactive cells did not differ between the two groups. Two months after infection, lymph nodes and spleens of transgenic mice contained more T_H1 and fewer T_H2 cells than did negative littermates (Fig. 4). Thus, prevention of the early burst of LACK-induced T_H2-like cytokines in IE-LACK transgenic mice appears to alter the initial cytokine milieu, redirecting the differentiation of other T cells along a T_H1 default pathway (*16*).

Variables in the initiation of T_H2 responses include the amount of priming antigen (*17*), the affinity or the determinant for MHC class II molecules (*18*), and the nature of the APCs (*19*). Although it remains to be determined if any of these parameters is responsible for the T_H2-biased response directed to LACK, it is very likely that this response has such a profound biological effect because it occurs within a critical early period when *Leishmania* promastigotes selectively inhibit IL-12 induction by macrophages (*20*). If the T_H2 response directed to LACK had occurred later, it would have had to compete with the amastigotes-induced IL-12, and its impact would not have been so strong (*5*).

Experimental crosses between different strains of mice have shown that a single locus (*Scl1*) on chromosome 11 is responsible for susceptibility or resistance to *L. major* infection (*21*). Adoptive transfer experiments have shown that both T cell and non–T cell compartments can independently determine resistance to *L. major* infection (*22*). In addition, a recent report has suggested that the susceptibility of BALB/c mice to

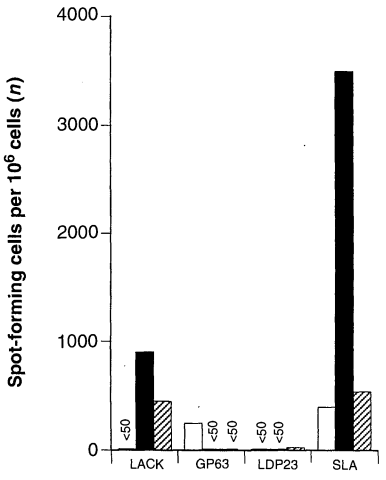

Fig. 1. Infection with *L. major* induces an early T_H2-polarized response against LACK. Female BALB/c mice 6 to 8 weeks old were infected with 2×10^6 stationary-phase promastigotes of *L. major* (strain WHOM/IR/-/173) by injection into each footpad. The frequency of T cells that secreted IFN-γ (open bars), IL-4 (solid bars), and IL-5 (dashed bars) in response to culture with LACK, GP63, LDP23, and SLA was determined for $CD4^+$ T lymph node cells by means of an ELISPOT assay (*11*). One of three experiments is shown. Data are means ± SD of triplicate independent determinations.

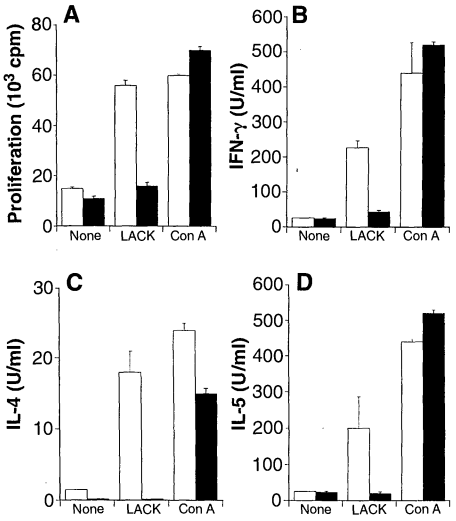

Fig. 2. IE-LACK transgenic mice are tolerant to LACK. IE-LACK (solid bars) and control littermates (open bars) were immunized with LACK in IFA. Ten days after immunization, lymph node cells were prepared and incubated with or without the LACK peptide (30 μM) or concanavalin A (2 μg/ml). (**A**) Proliferation and (**B**) IFN-γ, (**C**) IL-4, and (**D**) IL-5 secretion of lymph node cells were measured (*15*). Data are means ± SD of triplicate independent determinations.

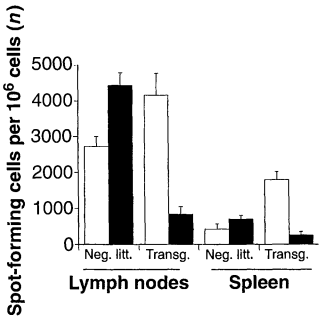

Fig. 4. IE-LACK tolerant mice developed a T_H1-dominated immune response. Eight weeks after infection, $CD4^+$ T cells were purified from the draining lymph nodes and the spleens of infected animals, and the frequency of T cells secreting IL-4 (solid bars) or IFN-γ (open bars) in response to SLA was determined by means of an ELISPOT assay (*11*). Data are representative of three separate experiments. Neg. litt., negative littermates; Transg., transgenic mice.

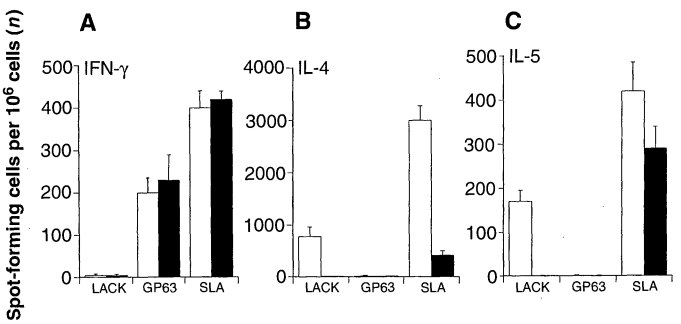

Fig. 3. Reduced T_H2 responses in IE-LACK transgenic mice after infection with *L. major*. $CD4^+$ T cells were purified from the draining lymph nodes of IE-LACK (solid bars) and negative littermates (open bars) at day 6 after infection. The frequency of T cells secreting (**A**) IFN-γ, (**B**) IL-4, and (**C**) IL-5 in response to LACK, GP63, and SLA was determined for the indicated antigens by means of an ELISPOT assay (*11*). One of three experiments is shown. Data are means ± SD of triplicate independent determinations.

infection with *L. major* may derive from the loss of the ability to generate IL-12–induced T_H1 responses rather than from an IL-4–induced T_H2 response (*23*). Here we propose that susceptibility to murine leishmaniasis, and possibly to other infectious diseases, is also determined by the ability of the infected host to mount a strong T_H2-polarized response against a restricted set of antigens. Thus, immune interventions aimed at modifying the T cell repertoire may be used to alter the course of an infection, as has been shown in the experimental treatment of autoimmune diseases (*24*). Tolerization protocols such as oral, intranasal, or intravenous administration of antigens (*25*) may be considered for down-regulation of deleterious immune responses and for conferring long-term protection against infectious diseases.

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10. Parasite-specific T cell clones were purified after incubation with SLA (50 μg/ml) and IL-2 (20 U/ml) as described (*26*). Reactivity to LACK was assessed by measurement of the amounts of IFN-γ and IL-4 released by each clone after incubation of 5×10^4 T cells with 2×10^6 mitomycin C–treated syngeneic splenocytes and SLA (50 μg/ml) or 30 μM LACK synthetic peptide (amino acids 158 through 173).
11. For the ELISPOT assay, 5×10^6 purified $CD4^+$ lymph node cells were activated in vitro with 5×10^6 mitomycin C–treated syngeneic splenocytes and SLA (50 μg/ml) or with an optimum concentration of LACK (amino acids 158 through 173) (FSPSLEHPIVVSGSWD) (*27*), GP63 (amino acids 364 through 378) (GSCTQRASEAHASLL), or LDP23 (amino acids 120 through 137) (RAHWARKVFDEKDAKTPV) synthetic peptides in 1 ml of Dulbecco's minimum essential medium. SLA was prepared as previously described (*28*). After culture for 72 hours, live cells were recovered by passage through Ficoll-Hypaque, washed, and transferred by serial dilution (from 10^5 to 5×10^3 cells per well) in triplicate to 96-well microtiter plates (Millipore, Bedford, MA) that had been coated with the capturing monoclonal antibodies (mAbs) (2 μg/ml), antibody to IFN-γ, antibody to IL-4, or antibody to IL-5. After 24 hours, cells were removed and spot-forming cells were visualized with biotinylated detecting mAbs and avidin D peroxidase in conjunction with 3-amino-9-ethylcarbazole (Sigma) substrate. Spots were counted under a dissecting microscope, and the frequency of antigen-specific cells was determined from the difference between the number of spots seen with and without antigen.
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15. Mice were immunized with 50 μg of recombinant LACK antigen in IFA. Ten days later, popliteal lymph node cells were prepared, and 2×10^6 cells were incubated in triplicate for 72 hours in a 96-well flat-bottomed plate with 30 μM LACK peptide, concanavalin A (2 μg/ml), or medium alone. Cells were pulsed with 1 μCi of [^{3}H]thymidine during the last 18 hours of culture. Plates were harvested and counted on a Betaplate Reader (Pharmacia, LKB). Parallel cultures were set up to measure amounts of cytokines in the supernatants by enzyme-linked immunosorbent assay, with the use of specific mAbs to IL-4, IL-5, or IFN-γ (PharMingen, San Diego, CA).
16. Because the IE-LACK transgenic mice were initially made on a B6/D2 F_1 background, we cannot completely rule out the possibility that the transgene integrated near and segregated with a resistant gene despite eight generations of backcrossing to BALB/c mice. This is, however, very unlikely to happen.
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Signaling by the *Escherichia coli* Aspartate Chemoreceptor Tar with a Single Cytoplasmic Domain per Dimer

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Many transmembrane receptors are oligomeric proteins. Binding of a ligand may alter the oligomeric state of the receptor, induce structural changes within the oligomer, or both. The bacterial aspartate chemoreceptor Tar forms a homodimer in the presence or absence of ligands. Tar mediates attractant and repellent responses by modulating the activity of the cytoplasmic kinase CheA. In vivo intersubunit suppression was used to show that certain combinations of full-length and truncated mutant Tar proteins complemented each other to restore attractant responses to aspartate. These results suggest that heterodimers with only one intact cytoplasmic domain are functional. The signaling mechanism may require interactions between dimers or conformational changes within a single cytoplasmic domain.

The aspartate chemoreceptor Tar of enteric bacteria forms a homodimer (*1*), each subunit of which has two transmembrane segments (TM1 and TM2). The Tar dimer forms a stable complex with a homodimer of the cytoplasmic histidine protein kinase CheA and two molecules of the cytoplasmic protein CheW (*2*). Binding of a repellent or an attractant activates or inactivates, respectively, CheA autophosphorylation and phosphotransfer to the cytoplasmic protein CheY. Phospho-CheY switches the direction of flagellar rotation to clockwise (*3*).

Aspartate binds to one of two sites at the subunit interface of the Tar homodimer (*4*, *5*). Because artificially disulfide–cross-linked dimers still function (*1*), signaling appears not to be mediated by a monomer-dimer transition. The TM1/TM1′ pair seems to be static (*4*, *6*, *7*), but TM2 has been proposed to move vertically, tilt, or rotate in response to aspartate binding (*6*, *8*). This movement could

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