istry and Biotechnology, H. J. Hinz, Ed. (Springer-Verlag, Berlin, 1986), pp. 402–427.
19. C. R. Cantor, P. R. Schimmel, *Biophysical Chemistry, Part III: The Behavior of Biological Macromolecules* (Freeman, San Francisco, 1980), pp. 1135–1139.
20. The type of "which-base-on-which-backbone" dependence of base-pairing strength encountered here seems to be a not uncommon feature of intersystem cross-pairing between complementary homobasic sequences belonging to different backbones. Although this was already apparent in the cross-pairing between RNA and DNA (Table 2) [see N. Sugimoto *et al.*, *Biochemistry* **34**, 11211 (1995); E. A. Lesnik, S. M. Freier, *Biochemistry* **34**, 10807 (1995)], it has been found to be a characteristic property of intersystem cross-pairing within the family of pentopyranosyl-(4′→2′)-oligonucleotides (*9*), and scattered literature information points to its occurrence in other combinations of base-pairing systems as well [see, e.g., M. J. Damha *et al.*, *J. Am. Chem. Soc.* **120**, 12976 (1998); A. Egger, C. Leumann, *Synlett* 913 (1999)]. Although conjectured to relate to steric differences between purines and pyrimidines in their ability to adjust nucleosidic torsion angles and, concurrently, the inclination between the backbone and base-pair axes that codetermines the topology of base-stacking (*9*), the phenomenon requires further study in order to be understood in detail.
21. T. Müller, postdoctoral report TSRI, 1998. Within the pentopyranosyl-(4′→2′) series, hydrolytic stability under these conditions parallels the occurrence of a *cis* relation between the free 3′-hydroxyl group and the adjacent phosphodiester groups.
22. In the stability tests referred to above, the DNA-sequence (T_8) was found to be completely unchanged after 8 days (monitoring was not pursued further).
23. For a recent experimental contribution to the question whether peptide nucleic acid (PNA) should be considered a potentially prebiotic type of structure see [K. E. Nelson, M. Levy, S. L. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3868 (2000); P. Garner, S. Dey, Y. Huang, *J. Am. Chem. Soc.* **122**, 2405 (2000)].
24. Studies on such template-directed synthesis of RNA oligonucleotides have recently been carried out by Orgel and colleagues [J. G. Schmidt, P. E. Nielsen, L. E. Orgel, *J. Am. Chem. Soc.* **120**, 4563 (1998); I. A. Kozlov, B. De Bouvere, A. Van Aerschot, P. Herdewijn, L. E. Orgel, *J. Am. Chem. Soc.* **121**, 5856 (1999); I. Kozler *et al.*, *Chem. Eur. J.* **6**, 151 (2000)] using Nielsen's PNA as well as Herdewijn's HNA (hexitol nucleic acid) and ANA (altritol nucleic acid) as template systems. Although the latter two are hexose-based oligonucleotides, they cannot be considered to be potentially natural nucleic acid alternatives according to the criteria given in (*1*). See also (*23*).
25. For pentofuranosyl systems with nitrogenous analogs of the phosphodiester bridge that have been shown to cross-pair with the natural systems, see [W. S. Zelinsky, L. E. Orgel, *Nucleic Acids Res.* **15**, 1699 (1987); S. M. Gryaznov *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5798 (1995); R. O. Dempcy, O. Almarsson, T. C. Bruice, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7864 (1994); N. Kojima, T. C. Bruice, *Org. Lett.* **2**, 81 (2000)].
26. One of the persistent weaknesses of the conventional scenario for the constitutional self-assembly of a prebiotic oligonucleotide base-pairing system is the necessity of assuming a spatial and temporal separation between the nitrogenous chemistry producing the nucleobases and the oxygenous chemistry supposed to give rise to carbohydrates. Drastically enhanced chemical complications would be expected for a scenario without that separation. A hypothesis that may conceptually overcome this complication with regard to backbone formation envisages the self-assembly of an informational oligomer system to result from combinatorial synthesis proceeding under (partial) thermodynamic control. The model considers monomer- and oligomer-formation steps to operate reversibly, yet to become eventually channeled towards oligomers that possess the capability of base pairing. Such oligomers could play off their bonus in relative thermodynamic stability by making base pairing the critical selection factor in the dynamic library of oligomers. Such a fragile "thermodynamic selection" by base pairing would have to be supplemented, and eventually superseded, by the more robust selection brought about by a target system's capability to replicate. Thermodynamic functional selection (by base pairing) would appear as a forerunner of kinetic functional selection (by replication), exemplifying on the chemical level one of biology's major lessons, namely, that replication can substitute for thermodynamic stability when continuance is at stake. The model would have to imply that a constitutionally labile replicator must be able to evolve to constitutionally more robust variants by undergoing a stepwise constitutional metamorphosis while retaining the system's specific informational capacity. The high kinetic lability to be assigned to a combinatorial reaction library should not be dismissed as being a priori lethal for a model of chemical self-organization. Quite the opposite may hold in the context under consideration: kinetically labile, rather than robust, reaction libraries can be expected to possess a high sensitivity toward emerging catalysts and, therefore, a susceptibility to becoming controlled by them.
27. Supported by The Skaggs Research Foundation. K.-U.S. and P.S. thank the Deutsche Forschungsgemeinschaft and S.G. thanks the NASA/NSCORT program for fellowship support. We thank S. Pitsch (ETH) for help and advice and D. Hilvert (ETH) for critically reading the manuscript and improving the English.

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Protection Against Cutaneous Leishmaniasis Resulting from Bites of Uninfected Sand Flies

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Despite the fact that *Leishmania* are transmitted exclusively by sand flies, none of the experimental models of leishmaniasis have established infection via sand fly bites. Here we describe a reproducible murine model of *Leishmania major* infection transmitted by *Phlebotomus papatasi*. Prior exposure of mice to bites of uninfected sand flies conferred powerful protection against *Leishmania major* that was associated with a strong delayed-type hypersensitivity response and with interferon-γ production at the site of parasite delivery. These results have important implications for the epidemiology of cutaneous leishmaniasis and suggest a vaccination strategy against this and possibly other vector-borne diseases.

The diseases transmitted by arthropod vectors afflict millions of people, particularly in developing countries. Bloodsucking arthropods may be more than just delivery systems for the pathogens they carry, insofar as components in their saliva have been shown to modify the outcome of infection (*1, 2*). Leishmaniasis is a vector-borne disease transmitted exclusively by sand fly bites. Reports of successful laboratory transmission of *Leishmania* spp. by sand fly bites are few (*3–8*) and have not addressed the host response to infective bites or considered the effects of prior exposure to uninfected sand fly bites on the outcome of infection. Using the murine ear model, we were able to transmit *Leishmania major* reproducibly to BALB/c and to C57BL/6 mice (*9*) by the bite of its natural vector, *Phlebotomus papatasi* (*10*). The respective healing and nonhealing phenotypes of C57BL/6 and BALB/c mice, established using high-dose needle inocula, were maintained in fly-transmitted infections. In BALB/c mice, nodular lesions increased steadily in diameter and thickness (Fig. 1, A and B). In C57BL/6 mice, the lesions increased in size up to day 70 when they began to resolve, with complete healing by day 120 (Fig. 1, C and D). Most of the lesions developed small focalized areas of ulceration prior to healing.

In endemic regions, many individuals are exposed to the bites of *Leishmania*-free phlebotomines before being bitten by an infected sand fly. For both BALB/c and C57BL/6 mice, prior exposure to *P. papatasi* bites (*11*) resulted in a striking reduction in the severity of the dermal lesions. For BALB/c mice, the difference between naïve and pre-exposed mice was significant from days 31 and 23 onward for lesion diameter and thickness, respectively ($P < 0.05$) (Fig. 1, A and B). For C57BL/6 mice (Fig. 1, C and D), the pre-exposed animals showed a delay in the appearance of lesions and a dramatic reduction in the peak lesion size that developed before healing. The attenuation in the diameter and/or thickness of lesions was significant during days 28 to 56 ($P < 0.05$). A second transmission experiment was undertaken in naïve and pre-exposed C57BL/6 mice to compare the parasite loads within the

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inoculation site. Two weeks after transmission by bite, the pre-exposed mice had over a 1000-fold reduction in the mean number of amastigotes present per infected ear ($P < 0.001$) (Fig. 2A), indicating that the protection conferred by uninfected fly bites was expressed relatively early after parasite delivery. As a relative measure of tissue parasite burden and as a biologically meaningful estimate of reservoir potential, a third set of C57BL/6 mice was used to investigate the ability of infected ears to transmit infections back to uninfected flies. When exposed to flies at the peak of lesion development, naïve mice provided a significantly better source of parasites than pre-exposed mice, with 11 of 14 ears (79%) able to transmit infections, as compared with 5 of 18 ears (28%) in the pre-exposed group (Fig. 2B). The percentage of blood-fed flies that picked up parasites averaged 27% per ear for the naïve mice and 10% for the pre-exposed mice ($P < 0.05$).

Prior sensitization to vector saliva could have impaired the feeding behavior of infected flies, compromising their ability to deliver parasites to the site. It is well documented that mammalian hosts develop acquired resistance to tick infestation as a result of previous exposure to tick bites (*12, 13*), which in turn confers host resistance to tick-transmitted pathogens such as *Borrelia* and *Pasteurella* spp. (*14*). However, for each of the transmission experiments described in the current studies, there was no significant difference in the number of blood-engorged flies recovered from the ears of naïve versus pre-exposed mice [Web table 1 (*15*)].

A mechanism of protection is suggested by a recent study involving needle inoculations of salivary gland sonicate (SGS) (*16*), in which the exacerbative effect of SGS on *L. major* infection, originally described by Titus and Ribeiro (*17*), was absent in mice that had been previously injected with SGS. The results shown here suggest that infective sand fly bites produce a similar outcome to the intradermal needle injection of a low dose of *L. major* metacyclics alone that is quite different from that produced by the coinoculation of parasites and SGS. The fly-transmitted infections in C57BL/6 mice in every instance resolved over time, similar to the injection of parasite alone (*16, 18*), whereas low-dose challenge in the presence of SGS produced nonhealing dermal lesions in C57BL/6 mice (*16*). In addition, the bites of infected *P. papatasi* did not elicit the early interleukin-4 (IL-4) response in the skin that was observed after the intradermal inoculation of SGS and *L. major*, being more comparable to the tissue response observed after the inoculation of parasites alone (*16*). These differences prompted us to investigate fly-transmitted infections in C57BL/6 IL-4–deficient mice (*9*), which along with mice treated with antibody to IL-4 have been shown to be refractory to the exacerbative effects of SGS (*16, 19*). The severity of the dermal lesions (Fig. 1, E and F) and the parasite loads in the site were not reduced in the IL-4–deficient mice as compared with wild-type mice, indicating that IL-4 is not required for, and has little effect on, the evolution of *L. major* infections in C57BL/6 mice after transmission by bite. The molecule(s) responsible for eliciting the early IL-4 response after inoculation of *P. papatasi* SGS may not be present in salivary secretions or may be deposited in the skin in much lower quantities during salivation. Similar discrepancies have been reported for black flies and mosquitoes, where salivary gland extracts elicited immune responses not observed after natural feeding (*20, 21*).

An adaptive immune response to sand fly saliva might modify the tissue environment into which the parasites are introduced in a manner that is directly harmful to the parasite. *Phlebotomus papatasi* bites produce a long-lasting delayed-type hypersensitivity (DTH) response in humans. Histologic analysis (*22*) of the ear dermis at 0, 3, 24, 48, and 72 hours after infective bite revealed a small, transient inflammatory reaction in naïve mice. In contrast, a large cellular infiltrate and a 200 to 300% increase in ear thickness that peaked at 24 hours and was sustained for up to 72 hours was seen in pre-exposed mice (Fig. 3A), which is indicative of a DTH response. In naïve mice, the leukocytes recovered from the entire ear dermis (*23*) 24 hours after fly bites were largely confined to neutrophils, contrasting with pre-exposed mice that showed a two- to fivefold increase in infiltrating neutrophils, eosinophils, macrophages, dendritic cells, and lymphocytes. This response was diminished by intraperitoneal injection of antibodies to CD4 24 hours before sand fly exposure [Web fig. 1 (*15*)]. The epidermal compartment in the mouse contains

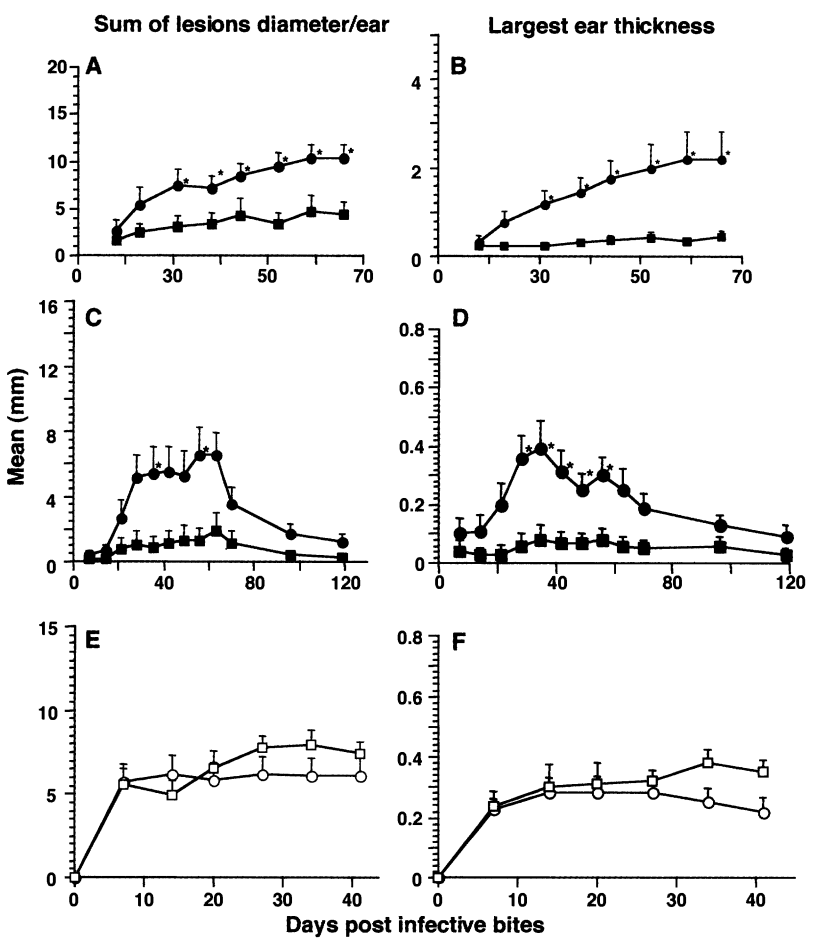


Fig. 1. The course of lesion development after transmission of *L. major* by bite of *P. papatasi*. Transmission by bite to ears of naïve (solid circles) and pre-exposed (solid squares) BALB/c mice (**A** and **B**) or of C57BL/6 mice (**C** and **D**) or to ears of naïve wild-type (open circles) and naïve IL-4–deficient (open squares) C57BL/6 mice (**E** and **F**) is shown. Mice were exposed to 10 infected flies on each ear. Ears were examined for the appearance of lesions at weekly intervals and were measured with a vernier caliper. Because multiple lesions tended to coalesce as they progressed, the values shown at each time point are the sum of the lesion's diameter (left panels) and the largest ear thickness (right panels), mean + 1 SE, 12 to 18 ears per group. Asterisks designate time points with values significantly different between naïve and pre-exposed groups ($P < 0.05$ by the two-tailed Student's t test) for each time point.

three major cell populations: keratinocytes, dendritic epidermal T cells (DETCs), and Langerhans cells, each of which can be activated to secrete a distinct set of cytokines (*24*). Six hours after exposure to infected flies, the epidermal cells recovered from naïve mice (*25*) consistently showed a strong up-regulation of IL-2 and IL-3 production (Fig. 3B). Smaller increases above steady-state frequencies were observed in cells producing interferon-γ (IFN-γ). Few cells stained for IL-4. The acute response of epidermal cells from pre-exposed mice was similar to that of naïve mice, with the clear exception of IFN-γ– and IL-12–producing cells (Fig. 3 B). The mean number of epi-

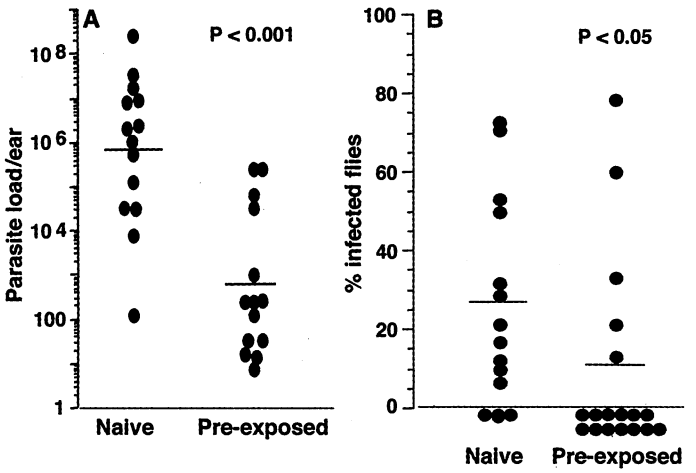
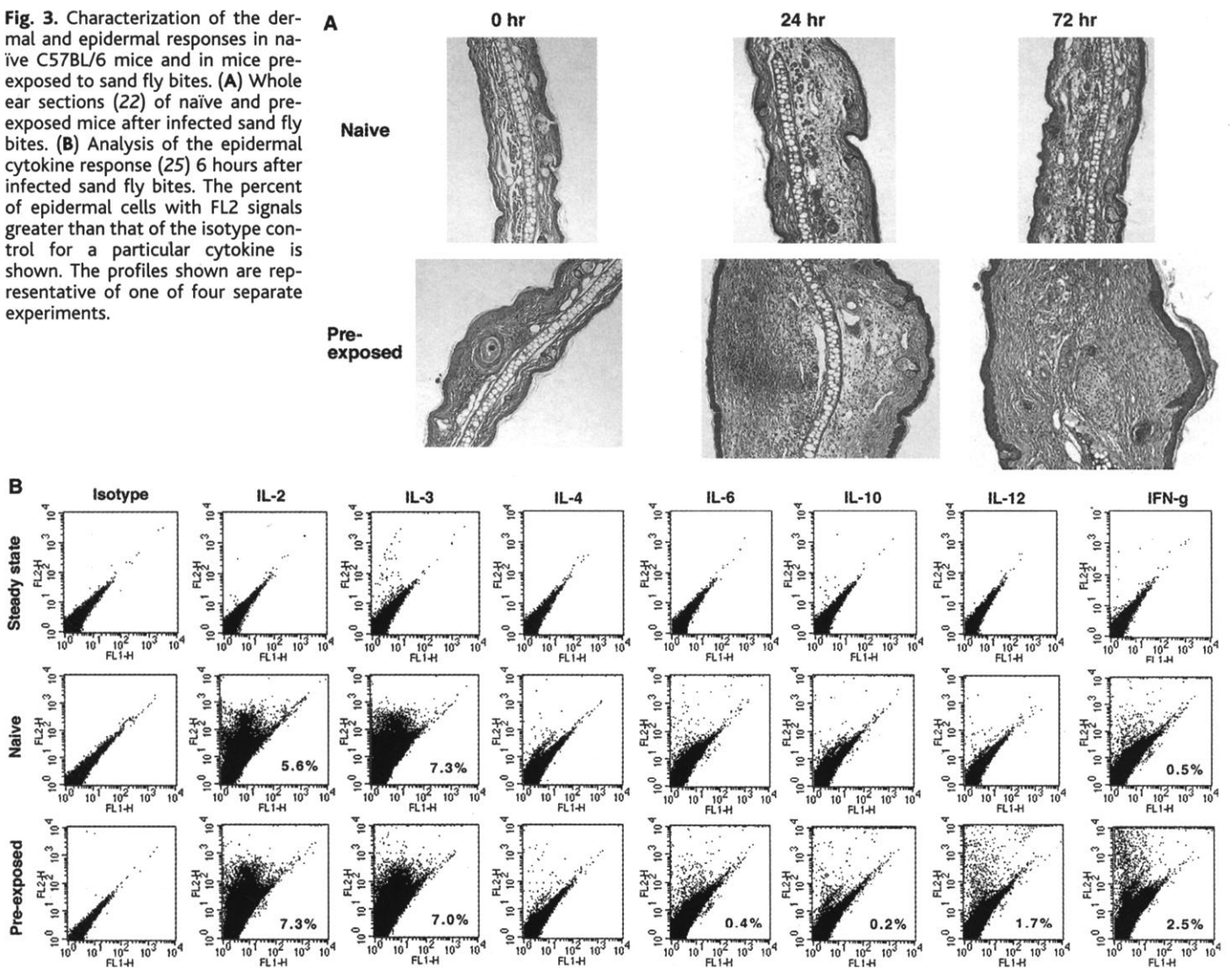


Fig. 2. Comparison of parasite loads and reservoir potential in naïve and pre-exposed C57BL/6 mice. (**A**) The number of viable amastigotes per ear titrated from complete tissue homogenates, obtained 14 days after transmission by bite. The number of parasites in exposed ears was determined by dilution to extinction of a homogenate of the entire ear dermis, prepared as described previously (*16*). Statistical significance was determined by the two-tailed Student's *t* test. (**B**) The transmissibility of *L. major* from infected ears to uninfected sand flies 48 days after transmission by bite. About 20 emergent female *P. papatasi* were allowed to feed for 1 to 2 hours on ears 7 weeks after fly-transmitted infection. Blood-fed females from each vial were separated and maintained in individual pots lined with plaster of Paris and were provided with a 50% sucrose and 5% albumin solution and water. Midguts were dissected 48 to 72 hours later and examined microscopically for the presence of promastigotes. Values represent the percent of blood-fed flies per ear that were positive for promastigotes. Statistical significance was determined by the one-tailed Student's *t* test.

Fig. 3. Characterization of the dermal and epidermal responses in naïve C57BL/6 mice and in mice pre-exposed to sand fly bites. (**A**) Whole ear sections (*22*) of naïve and pre-exposed mice after infected sand fly bites. (**B**) Analysis of the epidermal cytokine response (*25*) 6 hours after infected sand fly bites. The percent of epidermal cells with FL2 signals greater than that of the isotype control for a particular cytokine is shown. The profiles shown are representative of one of four separate experiments.



dermal cells producing IFN-γ and IL-12 per ear in pre-exposed mice was 1.02×10^5 and 3.2×10^4, which are 9- and 15-fold greater than in naïve mice, respectively. Langerhans cells and keratinocytes are important sources of IL-12 in the skin (*24, 26*), and each of the major epidermal populations has been shown to produce IFN-γ (*24, 27, 28*). The early and increased production of IFN-γ and IL-12 in the inoculation site may activate infected macrophages for killing during the initial establishment of infection and may also promote a more rapid and polarized *Leishmania*-specific T helper cell type 1 response.

The strong DTH response elicited at the inoculation site in mice previously exposed to sand fly bites conferred immunity against *L. major* infection that was as potent as any achieved by the combination of parasite antigens and adjuvants used to date. These findings imply that the exposure history of individuals to the bites of uninfected sand flies influences the incidence and severity of cutaneous leishmaniasis and that for this and possibly other vector-borne diseases, salivary antigens might be effective components of vaccines directed against transmitted pathogens.

References and Notes

1. J. M. C. Ribeiro, *Exp. Parasitol.* **69**, 104 (1989).
2. S. K. Wikel, *BioScience* **49**, 311 (1999).
3. R. Beach, G. Kiilu, J. Leeuwenburg, *Am. J. Trop. Med. Hyg.* **34**, 278 (1985).
4. E. Pozio, M. Maroli, L. Gradoni, M. Gramiccia, *Trans. R. Soc. Trop. Med. Hyg.* **79**, 524 (1985).
5. R. Lainson, R. D. Ward, J. J. Shaw, *Nature* **266**, 628 (1977).
6. R. Killick-Kendrick, A. J. Leaney, P. D. Ready, D. H. Molyneux, *Proc. R. Soc. London* **196**, 105 (1977).
7. P. G. Lawyer, D. G. Young, *J. Med. Entomol.* **24**, 458 (1987).
8. P. G. Lawyer *et al.*, *Trans. R. Soc. Trop. Med. Hyg.* **84**, 229 (1990).
9. Female BALB/c and C57BL/6 mice, aged 8 to 12 weeks, were purchased from the Division of Cancer Treatment, National Cancer Institute, Frederick, MD. IL-4–deficient mice, generated from an C57BL/6 embryonic stem cell line, were provided by N. Noben-Trauth.
10. Two- to 4-day-old *P. papatasi* females were obtained from a colony initiated from field specimens collected in Saudi Arabia. Flies were infected by artificial feeding on a chick membrane at 37°C. The bloodmeal consisted of heparinized mouse blood drawn intracardially from BALB/c mice, plus penicillin (100 U/ml) and streptomycin (100 μg/ml) to which 2×10^6 to 3×10^6 *L. major* amastigotes (MHOM/IL80/Friedlin) clone V1 were added. The amastigotes had been harvested from BALB/c footpads and cryopreserved in liquid nitrogen. Blood-fed flies were separated the following day and kept in an incubator at 25° to 26°C and 85 to 95% relative humidity. They were fed on a solution of 50% sucrose, 5% albumin, and sterile water through soaked cotton balls. The majority of infected flies showed a heavy parasite load (mean number, 60,000), a blocked stomodeal valve, and a high proportion of metacyclic promastigotes (≥30%) on days 14 to 15 after infection, when they were used for transmission. Flies were placed in plastic vials (volume, 3 drams; height, 4.8 cm; diameter, 1.8 cm) covered at one end with a 0.25-mm nylon mesh. Mice were anesthetized by intraperitoneal injection of 200 μl of ketamine hydrochloride (20 mg/ml) (Phoenix Pharmaceuticals, St. Joseph, MO). Individual ears of anesthetized mice were pressed flat against the mesh surface with specially designed clamps that held the vial and ear in place between support arms adapted at the ends with a flat rubber surface. The mice were also restrained by tape across the tail and back. Five or 10 infected flies were used per ear, and they were allowed to feed for 2 to 3 hours in the dark, after which time each fly was examined for blood.
11. The flies used for the pre-exposure of mice to the bites of uninfected sand flies were emergent females, left without sugar or water, and used the following day. For pre-exposure, flies were left to feed on both ears (10 to 15 flies per ear) for a period of 30 to 40 minutes in the dark, after which time at least five flies per ear became fully engorged with blood. Mice were exposed twice to uninfected flies at biweekly intervals.
12. M. Brossard, S. K. Wikel, *Med. Vet. Entomol.* **11**, 270 (1997).
13. S. K. Wikel, *Int. J. Parasitol.* **29**, 851 (1999).
14. P. Willadsen, F. Jongejan, *Parasitol. Today* **15**, 258 (1999).
15. Web table 1 and Web fig. 1 are available at *Science* Online at www.sciencemag.org/cgi/content/full/290/5495/1351/DC 1.
16. Y. Belkaid *et al.*, *J. Exp. Med.* **188**, 1941 (1998).
17. R. G. Titus, J. M. Ribeiro, *Science* **239**, 1306 (1988).
18. Y. Belkaid *et al.*, *J. Immunol.* **165**, 969 (2000).
19. M. L. Mbow, J. A. Bleyenberg, L. R. Hall, R. G. Titus, *J. Immunol.* **161**, 5571 (1998).
20. M. L. Cross, M. S. Cupp, E. W. Cupp, F. B. Ramberg, F. J. Enriquez, *J. Med. Entomol.* **30**, 725 (1993).
21. N. S. Zeidner, S. Higgs, C. M. Happ, B. J. Beaty, B. R. Miller, *Parasite Immunol.* **21**, 35 (1999).
22. Complete ears were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Thin sections (5 μm) were stained with hematoxylin and eosin. The transverse section showing the greatest thickness was measured with an ocular micrometer.
23. To obtain cells from a homogenate of the entire ear dermis, the sheets were separated and placed dermal side down in Dulbecco's modified Eagle's medium (DMEM) containing penicillin (100 U/ml), streptomycin (100 μg/ml), and collagenase A (1 mg/ml). After a 2-hour incubation at 37°C, the dermal sheets were cut into small pieces, filtered through a 70-μm nylon cell strainer, and rinsed twice in RPMI containing 20% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). The first rinse included 0.05% deoxyribonuclease. The leukocyte subpopulations were identified by characteristic size and granulosity, combined with two-color analysis as previously described (*16, 29*). For each sample, 10,000 cells were analyzed with CELLQuest software (Becton Dickinson, San Jose, CA).
24. A. Takashima, P. R. Bergstresser, *Semin. Immunol.* **8**, 333 (1996).
25. Epidermal cells were isolated from unbitten ears and from ears 6 hours after exposure to infected *P. papatasi*, according to a method recently described (*16*). Briefly, the epidermis was separated from the dermis after a 30-min incubation of the dermal sheets in DMEM and 0.5 to 0.1% trypsin at 37°C. The epidermal cells were collected through a 70-μm cell strainer, distributed in six-well plates, and incubated in Brefeldin A (Sigma) for 6 hours at 37°C. The cells were fixed in 4% paraformaldehyde and incubated in a permeabilization buffer consisting of antibody to Fc receptor in phosphate-buffered saline containing 10% normal mouse serum and 0.1% saponin. The cells were then washed and incubated with a battery of antibodies to cytokine and their isotype controls, each directly conjugated to R-phycoerythrin (Pharmingen), as specified previously (*16*). The stained cells were fixed again in 1% paraformaldehyde and washed, and for each sample, 100,000 cells were analyzed with CELLQuest software.
26. K. Kang *et al.*, *J. Immunol.* **156**, 1402 (1996).
27. A. Fayyazi *et al.*, *Arch. Dermatol. Res.* **291**, 485 (1999).
28. M. Sugaya, K. Nakamura, K. Tamaki, *J. Invest. Dermatol.* **113**, 350 (1999).
29. Y. Belkaid, H. Jouin, G. Milon, *J. Immunol. Methods* **199**, 5 (1996).

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Regulation of Antigen-Specific CD8$^+$ T Cell Homeostasis by Perforin and Interferon-γ

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T cell memory depends on factors that regulate expansion and death of these cells after antigenic stimulation. Mice deficient in perforin and interferon-γ (IFN-γ) exhibited increased expansion, altered immunodominance, and decreased death of antigen-specific CD8$^+$ T cells after infection with an attenuated strain of *Listeria monocytogenes*, which was cleared from these mice. Expansion of CD8$^+$ T cells was controlled by perforin, whereas IFN-γ regulated immunodominance and the death phase. Thus, perforin and IFN-γ regulate distinct elements of CD8$^+$ T cell homeostasis independently of their role as antimicrobial effector molecules.

In response to infection, CD8$^+$ T cells expand and differentiate into effector cells, which mediate pathogen clearance through perforin-dependent cytolysis or elaboration of IFN-γ or tumor necrosis factor (TNF) (*1*). The magnitude of expansion varies, depending on the infection, and reproducible hierarchies of immunodominance are observed in pathogen-specific CD8$^+$ T cell responses. After pathogen clearance, antigen-specific (Ag-specific) CD8$^+$ T cells undergo a reproducible death phase in which 80 to 90% of the expanded CD8$^+$ T cells are eliminated, irrespective of the magnitude of the initial expansion. The remaining Ag-specific memory CD8$^+$ T

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