Adoptive Immunotherapy against Experimental Visceral Leishmaniasis with CD8+ T Cells Requires the Presence of Cognate Antigen

Rosalind Polley,1†‡ Simona Stager,1†§ Sara Prickett,2‖ Asher Maroof,1§ Soomubl Zubairi,1§ Deborah F. Smith,2§ and Paul M. Kaye 1*

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT United Kingdom,1 and Wellcome Trust Laboratories for Molecular Parasitology, Department of Biological Sciences, Imperial College London, London, SW7 2AZ United Kingdom2

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CD8+ T cells have a protective role in experimental visceral leishmaniasis. However, the observation that inflammatory cytokines induce bystander activation of CD8+ T cells questions the need for antigen-dependent effector function. Here, we demonstrate that successful adoptive immunotherapy with CD8+ T cells is strictly dependent upon the presence of cognate antigen.

CD8+ T cells provide a major component of host defense against a variety of viral and nonviral pathogens and have been implicated as a major component of vaccine-induced immunity against some of the most important human pathogens (1–3). CD8+ T cells have been demonstrated to play a role in various experimental models of leishmaniasis, including infection with Leishmania major (2, 13), L. mexicana (10), L. infantum (21), and L. donovani (8, 14, 19, 20). However, the study of CD8+ T-cell function in leishmaniasis has been hampered compared to the study of their role in viral and bacterial infections by the lack of suitable tools for directly measuring the function of Leishmania-specific CD8+ T cells and for discriminating between antigen-dependent and potentially antigen-independent effector functions (10). In two models of cutaneous leishmaniasis, antigen-specific CD8+ T cells generated to defined Leishmania-expressed antigens have failed either to exert protection in vivo (22) or to kill infected macrophages in vitro (10). Recent studies demonstrating that a variety of inflammatory cytokines can mediate antigen-independent “bystander activation” of CD8+ memory and effector T cells, leading to host protection in different disease models (3, 6, 9), now question whether the effector function of CD8+ T cells in experimental leishmaniasis requires the presence of cognate antigen. Here, we have used adoptive CD8+ T-cell immunotherapy during experimental visceral leishmaniasis to directly examine this question.

* Corresponding author. Present address: Immunology and Infection Unit, Dept. of Biology, University of York, P.O. Box 373, York, YO10 5YW United Kingdom. Phone: 44 1904 324480. Fax: 44 1904 328845. E-mail: pmk2@york.ac.uk.
† These authors contributed equally to this work.
‡ Present address: Immunology and Infection Unit, Dept. of Biology, University of York, P.O. Box 373, York, YO10 5YW United Kingdom.
§ Present address: Immunology and Infection Unit, Dept. of Biology, University of York, P.O. Box 373, York, YO10 5YW United Kingdom.
†§ Present address: Division of Biomedical Sciences, Johns Hopkins in Singapore, 31 Biopolis Way, 02-01 The Nanos, Singapore 138669, Republic of Singapore.
‖ Present address: Division of Infection and Immunity, Walter and Eliza Hall Institute, 1G Royal Parade, Parkville, Victoria 3050, Australia.

We recently described a vaccination model in which host protection against L. donovani is mediated at least in part by CD8+ T cells with specificity for an amastigote-expressed antigen, hydrophilic acylated surface protein B1 (HASPB1) (18). HASPB1 is unusual in being targeted for expression at the plasma membrane by a unique N terminus which specifies its dual acylation (4), and this property may facilitate phagosomal presentation for class I processing (5). We therefore made use of this property of HASPB1 and generated transgenic L. donovani expressing a HASP1,18-OVA fusion protein. Full details of the generation of these and other Leishmania lines expressing HASP1,18-OVA will be published elsewhere. Briefly, the sequence encoding the N-terminal 18 amino acids of the HASPs (4) was cloned in frame with the OVA open reading frame and inserted into the pSSU vector for gene targeting by homologous recombination into a ribosomal DNA locus (11). Log-phase promastigotes of L. donovani (LV9) were electroporated with 40 μg of PacI/PmeI-digested DNA, and the resulting transfectants were maintained in 40 μg/ml hygromycin (Invitrogen) prior to single-cell cloning. Clones were screened for integration of the linear targeting fragment by Southern blot analysis of genomic DNA and for OVA expression by immunoblotting analysis of parasite lysate (Fig. 1). These transgenic parasites are formally designated Ld18SrRNA::HASP1,18-OVA (herein referred to as PINK). In order to recover infectivity, positive PINK clones were transformed to amastigotes (16) and maintained by serial passage in hamsters. Expression of OVA was maintained over several months of passage in the absence of drug selection. Semi-quantitative analysis of the levels of OVA expression indicated that these transgenic parasites expressed approximately 2 ng OVA/106 promastigotes or approximately 0.05% of the total amount of protein, similar to the expression level of HASPB in Leishmania (4) and somewhat higher than the level of OVA expressed in Leishmania from the episomal vector pX (7). Clones of transgenic OVA-expressing L. donovani were fully infective to mice, and host immunity was similarly organ specific, as has been described for wild-type infections. Clone 6, with maximal expression of OVA as determined by immunoblotting, was chosen for in vivo studies.
To determine whether adoptive immunotherapy with CD8 T cells altered the course of experimental visceral leishmaniasis and to define whether such an effect required in vivo antigen recognition, B6.CD45.1 mice were infected intravenously with $2 \times 10^7$ wild-type L. donovani (LV9) amastigotes or transgenic PINK amastigotes. At day 21 postinfection, adoptive transfer of $1 \times 10^6$ T-cell receptor transgenic OVA-specific OT-I CD8 T cells was performed (see below). Hepatic and splenic parasite burdens were determined by examining methanol-fixed, Giemsa-stained tissue impression smears 7 days after transfer (24). All animal procedures were approved by the LSHTM Animal Procedures Ethics Committee and the United Kingdom Home Office.

In the first series of experiments, we wished to evaluate whether in vitro-generated cytotoxic CD8 T cells were host protective. OT-I cytotoxic T lymphocytes (CTL) were generated by incubation of naïve OT-I cells with 1 nM OVA257–264, 350 ng/ml recombinant interleukin 4 (rIL-4), and 10 ng/ml rIL-2 for 3 days, followed by a further 2 days with cytokine and no peptide (1). CTL activity was confirmed by lysis of EG7-OVA (12) but not parental EL4 cells (data not shown). As shown in Fig. 2, the transfer of OT-I CTL into mice infected with wild-type L. donovani had no impact on parasite growth in either the liver or the spleen. In contrast, OT-I cells exerted significant control over the course of infection with PINK amastigotes in both target tissues. In the liver, this was evident as leishmanicidal activity, whereas in the spleen, the effect was restricted to being leishmanistatic. Together, these data both indicate a requirement for cognate antigen for successful immunotherapy by CTL and, by implication, demonstrate that the inflammatory environment created by wild-type L. donovani infection is insufficient to induce bystander effector responses from these CTL.

In the second series of experiments, we confirmed and extended these findings to other populations of CD8 T cells. CD8 memory T cells have been divided into two subsets. Effector memory T cells continually recirculate through peripheral tissues, whereas central memory T cells, by virtue of their high levels of expression of CD62L and CCR7, are endowed with lymph node homing capacity (17). CD8 T cells resembling effector and central memory cells were derived in vitro as described previously (23). Briefly, splenocytes from naïve OT-1 transgenic mice were incubated with 10 ng/ml OVA257–264 for 1 h at 37°C, washed, and cultured for a further 9 days with either 20 ng/ml rIL-2 (effector memory-like OT-I IL-2) or 20 ng/ml rIL-15 (central memory-like OT-I IL-15). Cytokines were changed every second day. OT-IIL-2 and OT-IIL-15 cells used for transfer were >88% CD62Llo and >75% CD62Lhi, respectively. In some experiments, cells were sorted on the basis of CD62L to >99% purity, with similar results.

As with OT-I CTL, neither OT-IIL-2 nor OT-IIL-15 cells had any effect on the course of wild-type L. donovani infection (Fig. 3A). In contrast, the transfer of OT-IIL-2 cells had a rapid
characteristics, as well as naı√ve OVA-specific CD8
Collectively, these data indicate that cells with memory cell
 cognition by endogenous CD8\(^+\) T cells during infection may be beneficial. Additional studies are required to confirm why bystander activation of effector function in CD8\(^+\) T cells does not occur to any significant extent in this model, in spite of extensive inflammation and evidence for bystander proliferation of both CD4\(^+\) (15) and CD8\(^+\) (15a) T cells. Furthermore, the model system that we describe here should also be useful for analyzing other facets of CD8\(^+\) T-cell biology, including the differential requirements for antigen-dependent activation of naïve versus effector/memory CD8\(^+\) T cells and the mechanisms underlying their protective effects.

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