Th1 Cell-Mediated Resistance to Cutaneous Infection with *Leishmania major* Is Independent of P- and E-Selectins[1]

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Studies in several models of inflammation have underscored the importance of P- and E-selectins in the migration of T cells to inflamed tissues. However, the role of the endothelial selectins in infection-induced cutaneous inflammation and host-protective immunity has not been investigated. In this study, we demonstrate that $CD4^+$ T cells recruited to the cutaneous compartment during infection with *Leishmania major* express P- and E-selectin ligands. Furthermore, expression of P- and E-selectin ligands correlates with activated *Leishmania*-specific Th1 cells and is dependent upon IL-12. To investigate the functional role of the endothelial selectins during leishmaniasis, we infected mice either singly or doubly deficient in the expression of P- and E- selectins. Mice lacking both P- and E-selectins developed significantly less inflammation at the site of a primary and secondary infection, and exhibited an impaired delayed-type hypersensitivity response. Surprisingly, the absence of the endothelial selectins had no effect on the control of parasite replication or immunity to reinfection. Thus, these data demonstrate that although the endothelial selectins contribute to the inflammatory response, they are not required for protective immunity to *L. major*. Moreover, these data suggest that by blocking P- and E-selectins, the immune pathology associated with cutaneous leishmaniasis might be ameliorated without compromising immunity to infection. ***The Journal of Immunology*, 2003, 171: 4726–4732.**

Lymphocyte migration is a sequential multistep process that involves rolling on inflamed endothelium, followed by integrin-mediated firm adhesion and ultimately transendothelial emigration (reviewed in Ref. 1). The initial adhesion event, critical to all subsequent steps, is due mainly to interactions between selectins on the endothelium and their ligands on circulating cells. The selectins recognize carbohydrate structures that are closely related to sialyl $Lewis^x$ presented on mucin-like molecules (2, 3). The three selectin family members, L-selectin (CD62L, expressed on lymphocytes), E-selectin (CD62E, expressed on endothelium), and P-selectin (CD62P, expressed on platelets and endothelium), have all been shown to mediate leukocyte rolling in vivo and in vitro (reviewed in Ref. 4). For lymphocytes, L-selectin is primarily involved in migration of naive cells to peripheral lymph nodes by interacting with its ligand, peripheral node addressin, on high endothelial venules (5–7). In contrast, P- and E-selectins are rapidly expressed on vascular endothelium after tissue trauma or activation by inflammatory mediators (including TNF-α, IL-1, and LPS) and bind to T cells expressing functional P- and E-selectin ligands (PESLs)[3] (8, 9).

The acquisition of selectin-binding epitopes by $CD4^+$ T cells is mediated primarily by the enzyme α(1,3)-fucosyltransferase VII (FucTVII) (10). FucTVII expression and activity are increased in Th1 cells and up-regulated by IL-12 in vitro (11–15). Previous studies have demonstrated that in vitro primed $CD4^+$ Th1 cells express PESLs and fail to migrate to inflamed skin in the presence of both anti-E- and anti-P-selectin Abs (16, 17). Thus, it is believed that migration of Ag-specific Th1 cells to sites of cutaneous inflammation is mediated by binding of the endothelial selectins to IL-12-induced, FucTVII-dependent epitopes on activated $CD4^+$ T cells.

Although the role of P- and E-selectins is clear in several models of acute inflammation (18–21), whether they are required for the recruitment of pathogen-specific Th1 cells and the development of protective immunity is unclear. *Leishmania major* is an obligate intracellular protozoan parasite that infects and survives in macrophages. Infection of mammals results in the development and resolution of a cutaneous lesion over several weeks (reviewed in Ref. 22). Animals that resolve infection, such as C57BL/6 mice, develop a dominant Th1 (characterized by production of IFN-γ) response, while susceptibility is associated with Th2 cells producing IL-4 and IL-13 (23, 24). The development of a Th1 response is dependent upon IL-12 that is produced by dendritic cells within the draining lymph node (dLN). Subsequently, control of parasite replication and resolution of the lesion require these $CD4^+$ Th1 cells to activate macrophages at the site of infection. Therefore, resistance to infection is dependent upon the expansion of *Leishmania*-specific $CD4^+$ Th1 cells in the dLN, followed by the migration of these cells to inflamed cutaneous tissues.

We hypothesized that the endothelial selectins would be required for optimal healing of a *L. major* infection. Surprisingly, we found that the endothelial selectins are dispensable for resistance to *L. major*. Selectin-deficient mice develop a polarized Th1 response, control parasite replication, and resolve their lesions. Moreover, not only were these animals able to resolve infection-induced inflammation, but mice deficient for both P- and E-selectins developed significantly smaller lesions than wild-type mice. Upon rechallenge, P- and E-selectin double knockout mice exhibited a defective delayed-type hypersensitivity (DTH) response, but nevertheless were able to control parasite replication as efficiently as wild-type mice. This study demonstrates that there is a critical balance between disease and protection mediated by Ag-specific

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[3] Abbreviations used in this paper: PESL, P- and E-selectin ligand; dLN, draining lymph node; DTH, delayed-type hypersensitivity; FucTVII, α(1,3)-fucosyltransferase VII; RPA, RNase protection assay; SLA, soluble *Leishmania* Ag.

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$CD4^+$ T cells. Thus, P- and E-selectins participate in the development of infection-induced pathology, but subsequent host-protective immunity can occur independently of the endothelial selectins.

Materials and Methods

Animals

C57BL/6J (B6), B6/129SF2/J (B6/129), B6;129S2-$Sele^{tm1Hyn}$ (E-$sel^{-/-}$), B6.129S7-$Selp^{tm1Bay}$ (P-$sel^{-/-}$), and B6;129S2-$Sele^{tm1Hyn}Selp^{tm1Hyn}$ (E/P-$sel^{-/-}$) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Groups of three to six female mice were used at 4–7 wk of age. For long-term experiments with E/P-$sel^{-/-}$ mice, only animals showing no signs of spontaneous oral infection were used (25). Animals were maintained in a specific pathogen-free environment and tested negative for pathogens in routine screening. All experiments were conducted following the guidelines of the University of Pennsylvania Institutional Animal Care and Use Committee.

Parasites and Ags

A clone of *L. major* (WHO/MHOM/IL/80/Friedlin) was used for these studies. Parasites were grown to stationary phase in Grace's insect cell culture medium (Life Technologies, Grand Island, NY) with 20% FBS (HyClone, Logan, UT; ≤0.125 EU/ml) and 2 mM glutamine (Sigma-Aldrich, St. Louis, MO). Stationary-phase promastigotes were harvested and washed three times in PBS. Soluble leishmanial Ag (SLA) was prepared, as described previously (26).

Courses of infection

For primary infections, mice were injected in the hind footpads with 5×10^6 stationary-phase *L. major* parasites. Footpad swelling was monitored using digital calipers (Mitutoyo, Kawasaki, Japan), and lesion size was determined by subtracting the size of the uninfected contralateral footpad from the size of the infected footpad. For secondary infections, healed mice (>12 wk postprimary infection) were rechallenged in the contralateral footpad with 2×10^6 stationary-phase *L. major* parasites. Secondary lesion size was determined by measuring the size of the infected footpad and subtracting the size of the footpad before infection. To quantify parasites in lesions, single-cell suspensions were prepared and plated in 10-fold serial dilutions (initial dilution of 1/100) in Grace's insect culture medium. Each sample was plated in quadruplicate, and the mean of the negative log parasite titer was determined after 7 days of culture at 26°C.

Cell culture and cytokine analysis

Draining popliteal lymph nodes were harvested, and single-cell suspensions were prepared in complete tissue culture medium (DMEM supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, and 5×10^{-5} M 2-ME). In some cases, cells were first stained with CFSE (Molecular Probes, Eugene, OR), as previously described (27, 28), and cultured at 2×10^5 cells/well in round-bottom 96-well plates. In all other experiments, cells were plated at 4×10^6/ml in 24-well culture plates in medium alone or in the presence of SLA (50 μg/ml). Supernatants were harvested after 72 h and assayed by sandwich ELISA using paired mAb to detect IL-4 (11B11 and BVD6-24G2.3) and IFN-γ (R46A2 and polyclonal rabbit anti-IFN-γ).

In some experiments, lymphocytes were isolated from lesions for analysis. After removing the skin from the infected footpad, lesions were homogenized in complete tissue culture medium in a 7-ml glass tissue homogenizer. Tissue homogenate was passed through a 70-μm mesh filter, and viable lymphocytes were purified using Lympholyte-M (Cedarlane Laboratories, Hornby, Ontario, Canada), following the manufacturer's recommendations.

Flow cytometry and intracellular cytokine staining

Cells isolated from dLNs were analyzed by flow cytometry for expression of surface markers directly ex vivo as well as cytokines after in vitro restimulation. Cells were washed in staining buffer (PBS containing 0.1% BSA and 0.1% sodium azide) and incubated with Fc block (50 μg/ml 2.4G2 and 500 μg/ml rat Ig) before incubation with specific fluorochrome-conjugated mAbs against CD4, CD62L, or isotype-specific control Abs (all from BD PharMingen, San Diego, CA). For intracellular cytokine staining, cells were stimulated with 50 ng/ml PMA, 500 ng/ml ionomycin, and 10 μg/ml brefeldin A (all from Sigma-Aldrich) for 4 h before surface staining. Fixed and surface-stained cells were permeabilized with 0.3% saponin (Sigma-Aldrich) in staining buffer before staining with specific fluorochrome-conjugated mAbs against IFN-γ or IL-4 (BD PharMingen).

PESL expression was measured by staining with P-selectin Ig (BD PharMingen) or E-selectin Ig (R&D Systems, Minneapolis, MN) chimeric molecules, followed by FITC or PE anti-human IgG (Jackson ImmunoResearch, West Grove, PA). In preliminary experiments, we did not see any differences in the binding of P- or E-selectin Ig molecules to $CD4^+$ T cells in vitro or in vivo (data not shown). Therefore, in most experiments, P-selectin Ig and E-selectin Ig chimeras were mixed together (1:1). Human CTLA-4-Ig (ChiL6) was used for control staining with selectin-Fc molecules.

RNase protection assay

Draining popliteal lymph nodes were removed and total RNA was extracted using RNAStat60 (Tel-Test, Friendswood, TX), according to the manufacturer's recommendations. Gene expression was measured using the RiboQuant RNase protection assay (RPA) system (BD PharMingen; custom probe containing FucTVII, IFN-γ, L32, and GAPDH), following the instructions of the supplier. The quantity of protected RNA was determined using a phosphor imager and MultiAnalyst software (both from Bio-Rad, Culver City, CA). For quantitation, normalized values are expressed as units relative to the L32 ribosomal RNA gene.

Statistics

Results represent the mean ± SEM, unless otherwise stated. Statistical significance was determined by the Student's *t* test, and results were considered significant with a p value less than 0.05.

Results

PESLs are preferentially expressed on $CD4^+$ T cells in lesions of infected mice

To determine whether $CD4^+$ T cells from resistant mice infected with *L. major* expressed PESLs after activation in vivo, the binding of P- and E-selectin Fc chimeric molecules to $CD4^+$ T cells from dLNs and lesions from 21-day-infected C57BL/6 mice was examined. Fig. 1 shows that directly ex vivo, only 6–8% of $CD4^+$ T cells in the dLN expressed PESLs. In contrast, greater than 50% of $CD4^+$ T cells isolated from lesions of infected mice expressed PESLs, which increased to greater than 80% by 5 wk postinfection (data not shown). Using forward scatter (a measure of cell size) as a marker of activation, large, activated $CD4^+$ T cells isolated from the lesions uniformly express PESLs. Thus, although only a small percentage of $CD4^+$ T cells in the dLN expressed PESLs ex vivo after infection with *L. major*, the $CD4^+$ T cells that accumulated at sites of cutaneous inflammation were predominantly PESL positive.

FucTVII expression is induced in the dLN after infection

Previous in vitro studies have shown that IL-12-dependent expression of FucTVII is required for the expression of PESLs on T cells

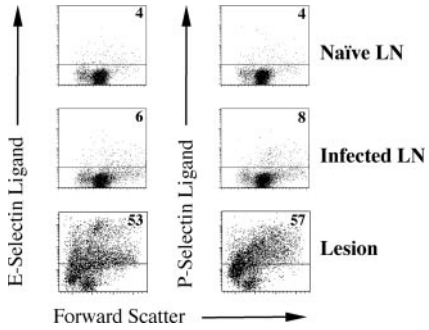

FIGURE 1. $CD4^+$ T cells from lesions express PESLs. Cells were isolated from the popliteal LNs of naive C57BL/6 mice, or from the popliteal LNs and lesions of C57BL/6 mice infected for 3 wk with *L. major*. The cells were examined by flow cytometry for expression of CD4 and binding to P- and E-selectin Ig chimeric molecules. Dot plots of $CD4^+$ lymphocytes are presented as P- or E-selectin Ig binding as a function of cell size (forward scatter), and numbers represent percentage of $CD4^+$ that bind to P- or E-selectin. Data are representative of three separate experiments.

(10–12, 29, 30). To investigate expression of FucTVII during leishmaniasis, cells were isolated from dLNs of 2-wk-infected C57BL/6 mice, total RNA was isolated, and gene expression was quantified by RPA. Although only 6–8% of $CD4^+$ T cells in the dLNs expressed PESLs, expression of the FucTVII gene was markedly increased by 14 days after infection with *L. major* (Fig. 2*A*). To test whether FucTVII expression following infection was IL-12 dependent, mice were infected and IL-12 was depleted 1 day before infection. Consistent with published results, blockade of IL-12 decreased expression of infection-induced IFN-γ (31). Furthermore, α-IL-12 treatment significantly reduced FucTVII expression after infection (Fig. 2*A*). Control-treated mice had a 3.3-fold increase in FucTVII gene expression over uninfected mice, while IL-12-depleted mice had only a 1.4-fold increase over naive mice (Fig. 2*B*). To confirm the gene expression data, cells isolated from α-IL-12-treated and untreated 2-wk-infected mice were cultured for 3 days in the presence or absence of SLA, and PESL expression was examined by flow cytometry (Fig. 2*C*). $CD4^+$ T cells isolated from α-IL-12-treated mice failed to up-regulate PESLs to the same extent as control mice after in vitro culture. Therefore, infection with *L. major* results in the IL-12-dependent up-regulation of FucTVII in the dLN, suggesting that the Ag-reactive T cells in the dLN have already begun to express FucTVII, a prerequisite for functional PESL expression.

PESL expression correlates with $CD4^+$ T cell activation and effector function

Cells isolated from dLNs of *L. major*-infected mice were stimulated with SLA and analyzed for activation and effector function by flow cytometry. With increased stimulation over time, we observed the accumulation of $CD62L^{low}$ $CD4^+$ T cells, a phenotype consistent with increased frequency of activated T cells (Fig. 3*A*).

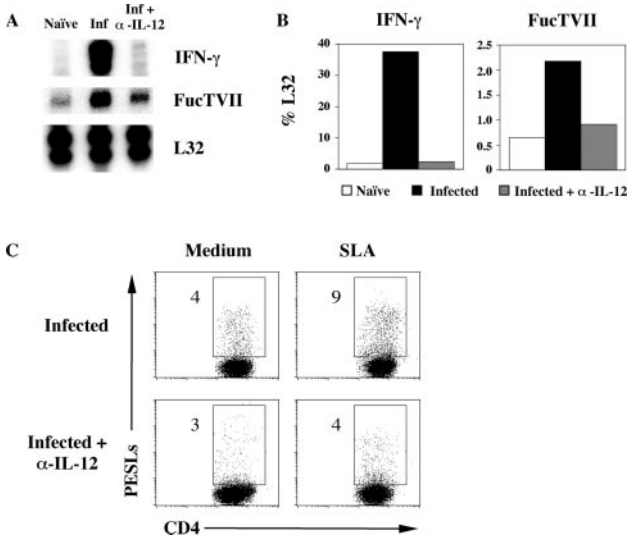

FIGURE 2. Infection-induced up-regulation of FucTVII in dLNs is IL-12 dependent. C57BL/6 mice were treated with either 1 mg of rat Ig or α-IL-12 (C17.8) 1 day before infection in the hind footpad with 5×10^6 stationary-phase *L. major* promastigotes. Cells were isolated from LNs of naive or 2-wk-infected C57BL/6 mice and examined by RPA for gene expression of FucTVII and IFN-γ directly ex vivo or by flow cytometry for expression of PESLs after in vitro restimulation. *A*, RPA autoradiograph of FucTVII, IFN-γ, and L32 expression levels. *B*, Graphical representation of data presented in *A*. *C*, Expression of PESLs after 3 days of in vitro culture. Numbers represent percentage of $CD4^+$ lymphocytes binding to P- and E-selectin Ig chimeric molecules. Data are representative of two independent experiments.

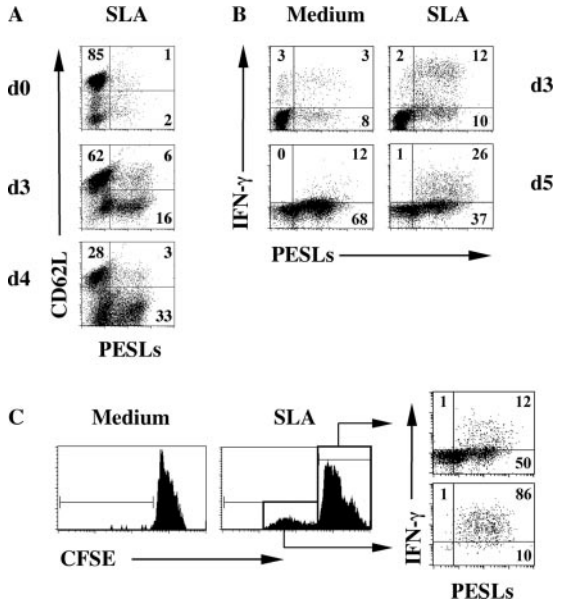

FIGURE 3. Activated Ag-specific IFN-γ-producing $CD4^+$ T cells express PESLs after proliferation. Cells isolated from dLNs of *A*, 2-wk-infected or *B* and *C*, 5-wk-infected C57BL/6 mice were cultured in vitro in the presence or absence of 50 μg/ml SLA. Some cells were stained with CFSE to monitor Ag-specific proliferation. After 2–5 days in culture, cells were examined by flow cytometry for expression of *A*, CD62L and PESLs; *B*, PESLs and IFN-γ production; or *C*, CFSE, PESLs, and IFN-γ. Dot plots are gated on $CD4^+$ cells. Numbers represent percentage of $CD4^+$ T cells in each quadrant. Data are representative of two independent experiments.

In addition, $CD4^+$ T cells expressed increasing levels of PESLs after in vitro restimulation (day 0, 3%; day 3, 22%; day 4, 36%). Furthermore, after 4 days of culture, greater than 90% of the $PESL^+$ population of $CD4^+$ T cells expressed low levels of CD62L. Thus, activation of Ag-specific $CD4^+$ T cells resulted in the acquisition of PESLs, as well as the coordinated down-regulation of CD62L. In terms of lymphocyte migration, this phenotype is associated with tissue-homing effector $CD4^+$ T cells.

To determine whether the loss of CD62L and expression of PESLs correlated with effector cytokine production, we analyzed cells isolated from dLNs of 5-wk-infected mice for IFN-γ production. There was a strong correlation between IFN-γ production and PESL expression, with greater than 85% of the IFN-γ-positive $CD4^+$ T cells being $PESL^+$ on days 3 and 5 after restimulation (Fig. 3*B*). Interestingly, PESL expression was not dependent upon proliferation, as >60% of nonproliferating $CD4^+$ T cells expressed PESLs after 5 days of Ag stimulation (Fig. 3*C*, *top*), and even in the absence of stimulation, there is an up-regulation of PESLs on $CD4^+$ T cells after 5 days of in vitro culture (Fig. 3*B*). In contrast, greater than 95% of the cells that had proliferated stained positive for PESL expression (Fig. 3*C*, *lower panel*). These results demonstrate that effector $CD4^+$ T cells, as measured by IFN-γ production, are largely PESL expressers.

Selectin-deficient mice are resistant to infection with L. major

Several findings have demonstrated a critical role for P- and E-selectins in $CD4^+$ T cell migration to the skin (16, 17, 30). Based on our data that a majority of $CD4^+$ T cells in the lesion were $PESL^+$ and that PESL expression correlates with T cell activation and IFN-γ production, we predicted that in the absence of P- and E-selectins, mice would exhibit defective recruitment of effector cells and impaired resistance. We first infected E-$sel^{-/-}$ and P-$sel^{-/-}$ mice with *L. major*. Recent experiments have suggested

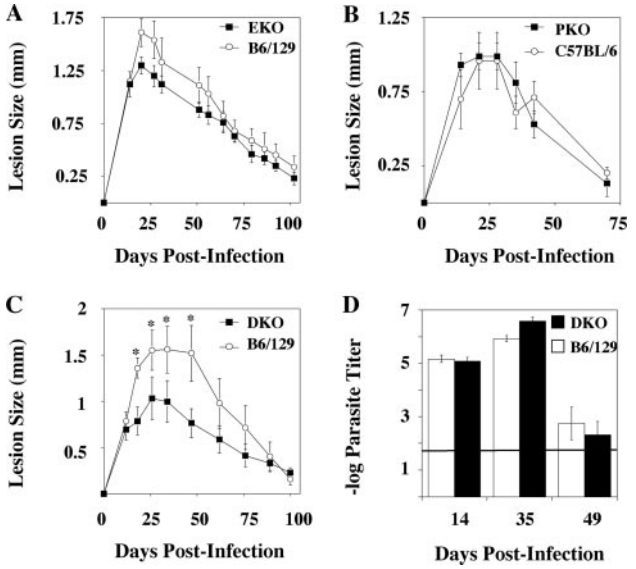

FIGURE 4. Selectin-deficient mice are resistant to infection with *L. major*. *A–C*, B6, B6/129, E-sel$^{-/-}$, P-sel$^{-/-}$, and E/P-sel$^{-/-}$ mice were infected in the hind footpad with 5×10^6 stationary-phase *L. major* promastigotes, and lesion development was monitored over the course of infection. Values represent mean lesion size in mm ± SEM from four to five mice per group. *D*, Parasite titer was measured by limiting dilution analysis. Results are presented as negative log of the lowest dilution that allowed parasite growth ± SEM with a minimum of three mice per group. The experiments shown are representative of three independent experiments. *, $p < 0.05$.

that E-selectin may be particularly important for migration of CD4$^+$ T cells to the cutaneous tissues (30). However, we found that both E-sel$^{-/-}$ and P-sel$^{-/-}$ mice developed lesions that were identical in size to wild-type animals and healed in 12 wk (Fig. 4, *A* and *B*). Furthermore, parasite numbers and cytokine responses were indistinguishable from wild-type mice during infection (data not shown). Thus, we concluded that if the endothelial selectins are important for resistance, there is compensation for the absence of either molecule, a result consistent with earlier studies (32).

To address the issue of compensation between the endothelial selectins, we next infected mice deficient in both P- and E-selectins with *L. major* and followed the course of infection. Following infection, E/P-sel$^{-/-}$ mice successfully resolved their lesions (Fig. 4*C*). However, in contrast to E-sel$^{-/-}$ or P-sel$^{-/-}$ mice, E/P-sel$^{-/-}$ mice developed significantly smaller lesions than wild-type controls (Fig. 4*C*). In three experiments, the overall average reduction in lesion size was 47 ± 5%. Such a reduction in lesion size could be due to a reduced number of cells infiltrating lesions, reduced numbers of parasites, or both. To examine this issue, parasite numbers in lesions were determined at several time points postinfection. We found that selectin-deficient mice were indistinguishable from wild-type mice in parasite titers at all time points examined (Fig. 4*D*). Thus, although the lesions at 5 wk postinfection in E/P-sel$^{-/-}$ mice were almost 50% smaller than those observed in wild-type mice, the parasite levels were the same, indicating that a reduction in cell infiltration was the likely cause of reduced lesion size. Histological examination of lesions demonstrated that although the size of lesions differed between wild-type and E/P-sel$^{-/-}$ mice, there were no gross differences in the cellular makeup of the lesions (data not shown). These results indicate that the endothelial selectins are dispensable for the development of resistance, but are involved in the infection-induced pathological response.

Selectin-deficient mice develop a polarized Th1 response, but have an impaired DTH

One of the hallmarks of immunity to infection with *L. major* is the development of a strong DTH response. Swelling due to DTH is a result of the influx of Ag-specific T cells into the site of challenge by 72 h after Ag deposition (33), and it has previously been demonstrated that E/P-sel$^{-/-}$ mice exhibit a defective DTH response in chemical-induced hypersensitivity models (18, 21) To examine the capacity of Ag-specific effector T cells generated during *L. major* infection to migrate to inflamed tissues, we tested healed E/P-sel$^{-/-}$ mice for DTH responses to *L. major*. We injected *L. major* parasites into the contralateral footpad of mice infected for 12 wk and measured swelling 72 h later. Wild-type mice, as well as mice singly deficient in either E- or P-selectin, exhibited a substantial DTH response, while E/P-sel$^{-/-}$ mice had a 50% reduction in DTH (Fig. 5*A* and data not shown). Thus, although E/P-sel$^{-/-}$ mice were able to control parasite replication, they were defective in their ability to mount a DTH response, suggesting that Ag-specific T cell migration was impaired in the absence of P- and E-selectins.

To confirm that the decreased DTH was due to defective T cell migration rather than a decrease in the generation of Ag-specific T cells, we analyzed the cytokine responses in wild-type and selectin-deficient mice. Cells isolated from the dLNs of 5-wk-infected E/P-sel$^{-/-}$ mice produced high levels of IFN-γ and low levels of IL-4 after in vitro Ag restimulation, comparable to control mice

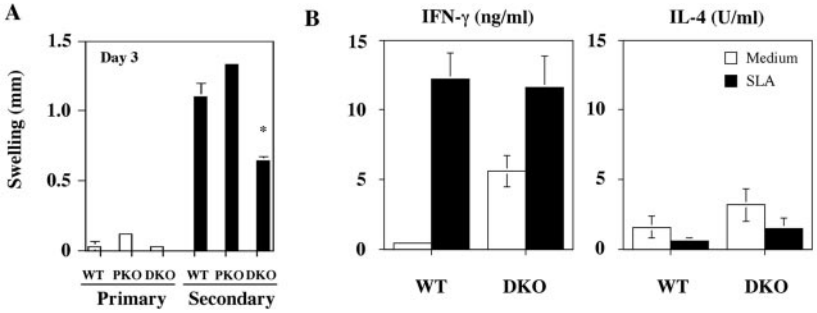

FIGURE 5. Selectin-deficient mice develop a polarized Th1 response, but have a defective DTH. *A*, Ag-specific DTH response was measured 72 h after challenge of healed mice (>12 wk postinfection) with 2×10^6 stationary-phase *L. major* promastigotes in the contralateral footpad. Results are presented as mean ± SEM of at least three individual mice and are representative of five independent experiments. *B*, Wild-type and E/P-sel$^{-/-}$ mice were infected in the hind footpad with 5×10^6 stationary-phase *L. major* promastigotes and sacrificed 5 wk postinfection. LN cells from infected mice were cultured in the presence or absence of 50 μg/ml SLA for 72 h. Supernatants were analyzed by ELISA for IFN-γ production. Results are presented as mean ± SEM of three individual mice and are representative of two separate experiments. *, $p < 0.05$.

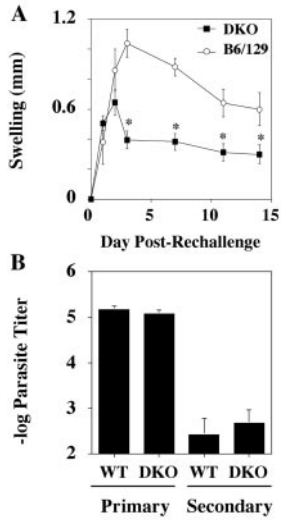

FIGURE 6. P- and E-selectins are required for infection-induced immunopathology, but dispensable for resistance. Healed wild-type or E/P-sel$^{-/-}$ mice (>12 wk infected) were rechallenged in the contralateral footpad with 2×10^6 stationary-phase *L. major* promastigotes, and footpad swelling was measured daily. *A*, Time course of footpad swelling after reinfection of the contralateral footpad. *B*, Parasite titer was determined by limiting dilution analysis. Results presented are the mean ± SEM of three to four individual mice from one representative experiment of three. ∗, $p < 0.05$.

(Fig. 5*B*). These results indicate that effector T cell development is intact in E/P-sel$^{-/-}$ mice during infection with *L. major*, and that decreased lesion size and DTH in E/P-sel$^{-/-}$ mice are due to impaired T cell migration.

Infection-induced resistance is intact in E/P-sel$^{-/-}$ mice

Mice that have resolved a primary infection with *L. major* are resistant to reinfection. Infection-induced resistance is characterized by a strong DTH response, accelerated lesion development, and rapid parasite clearance. The observed defect in DTH in E/P-sel$^{-/-}$ mice (Fig. 5*B*) suggested that E/P-sel$^{-/-}$ mice might have impaired resistance to rechallenge. To investigate this, E/P-sel$^{-/-}$ and wild-type mice that had resolved lesions following a primary infection were infected in the contralateral footpad, and lesion development was monitored over 2 wk. In wild-type mice, there is a rapid swelling of the site of a challenge infection that is due to effector cell migration into the tissues rather than parasite growth. In contrast, E/P-sel$^{-/-}$ mice developed significantly smaller lesions (48 ± 2% reduction in mean peak lesion size) during a secondary infection (Fig. 6*A*), indicative of either a reduced effector cell response to challenge or defective recruitment. Nevertheless, analysis of parasite burden at 2 wk postchallenge revealed that E/P-sel$^{-/-}$ mice controlled infection as effectively as wild-type mice (Fig. 6*B*). Thus, the absence of the endothelial is associated with a significant reduction in lesion size in both a primary and secondary infection, but has no effect on the ability to eliminate parasites in cutaneous lesions.

Discussion

A role for the endothelial selectins in the migration of T cells to extralymphoid tissues has been reported in several murine models of inflammation and immunity to infectious pathogens (16, 18–21, 34–37). In this study, we examine the role of P- and E-selectins in the development of immunity and pathology during the immune response against the intracellular pathogen, *L. major*. Surprisingly, our study shows that P- and E-selectins are not required for the expression of host-protective immunity against cutaneous leishmaniasis. Conversely, we found that in the absence of the endothelial selectins, infection-induced pathology is significantly reduced during both primary and secondary immune responses. A significant part of the destruction caused by cutaneous leishmaniasis is due to severe inflammation at the site of infection, and these data provide compelling evidence that by blocking P- and E-selectins, this immunopathology may be ameliorated without compromising resistance.

Acquisition of tissue-homing capacity by T cells is thought to begin after activation in the lymph node draining a site of Ag challenge or infection (38). Naive T cells migrate to LNs based primarily on expression of L-selectin (CD62L), which is downregulated after T cells encounter their cognate Ag and proliferate. T cell activation leads to increased expression of FucTVII, which posttranslationally fucosylates P-selectin glycoprotein ligand 1 to create functional PESLs (10, 13, 14). Activated (CD62L^{low}) T cells exit the LN and migrate via the circulation. T cells expressing the correct ligands for molecules expressed on inflamed endothelium tether and roll (mediated by selectin/selectin ligand interactions), firm adhere (mediated by interactions between integrin and Ig-superfamily molecules), and transmigrate into the inflamed tissues. Our data are consistent with this model, as we observed that effector Th1 cells isolated directly from lesions of infected mice express high levels of PESLs. Furthermore, there was a striking association among proliferation, IFN-γ production, and PESL expression by *Leishmania*-specific CD4$^+$ T cells, suggesting that cytokine expression and acquisition of tissue-homing properties are linked during T cell activation and differentiation in vivo.

The expression of functional selectin ligands by CD4$^+$ T cells is dependent upon the activity of FucTVII (10, 13, 29, 39). Consistent with previous in vitro studies (11, 12), we demonstrate for the first time that IL-12 regulates FucTVII gene expression and PESL expression in vivo. Thus, during infection with *L. major*, infection-induced IL-12 mediates not only IFN-γ production by CD4$^+$ T cells, but also directs the acquisition of PESLs that are involved in the migration of effector Th1 cells to tissues.

Austrup et al. (16) were the first to demonstrate a role for P- and E-selectins in the migration of effector Th1 cells to inflamed cutaneous tissues, while a recent report has shown a clear role for E-selectin, but not P-selectin, in the migration of effector CD4$^+$ T cells to peripheral tissues (30). Furthermore, it has previously been reported that mice infected with *L. major* up-regulate expression of E-selectin on postcapillary venules as early as 6 h postinfection (40). Thus, we hypothesized that selectin/selectin ligand interactions (especially E-selectin) would be critical to the development of resistance to challenge with *L. major*. However, we were surprised to find that the endothelial selectins were dispensable for resistance to infection with *L. major*. Mice singly deficient in either P- or E-selectin were identical with wild-type mice in the development and resolution of their lesions, consistent with studies in which individual selectin knockout animals have mild phenotypes when compared with the defects of animals deficient in both P- and E-selectins (18, 19, 25, 32). In fact, all selectin-deficient mice, including E/P-sel$^{-/-}$ mice, were indistinguishable from wild-type animals in control of parasite replication and cytokine responses throughout the course of infection. However, the absence of both of the endothelial selectins had a significant effect on the development of infection-induced inflammation in the cutaneous tissues without disrupting immunity. The development of smaller lesions was not associated with any gross histological changes during the course of infection, suggesting that the absence

of the selectins affected the quantitative, but not qualitative, migration of lymphocytes to inflamed tissues. Thus, our study demonstrates that while P- and E-selectins facilitate T cell migration to skin, they are not absolutely required.

DTH has long been associated with the development of resistance to leishmaniasis and other diseases, such as tuberculosis and leprosy (41). It has often been used as a correlate of immunity in vaccine trials (42) and is considered predictive of successful chemotherapy in visceral leishmaniasis (43). In mice, rechallenge of healed *L. major*-infected mice results in the development of a strong tuberculoid-type DTH response (44) that is mediated by $CD4^+$ T cells (45). However, whether DTH is required for infection-induced immunity is not known (44, 46, 47). The endothelial selectins have been shown to be a critical component of the contact hypersensitivity response to 2,4-dinitro-1-fluorobenzene or oxazolone, chemical models of DTH (18). Similarly, we demonstrate that E/P-$sel^{-/-}$ mice have an impaired DTH response after rechallenge and decreased pathology immediately following rechallenge. Most importantly, this defect in DTH and pathology did not affect immunity to rechallenge, indicating that a strong DTH response is not an absolute requirement for infection-induced immunity. Taken together, these results suggest that although selectin-selectin ligand interactions are critical for immunopathology, they are dispensable for resistance to infection with *L. major* and may in fact promote infection-induced pathology associated with leishmaniasis.

The ability to control pathological infection-induced inflammation without disrupting immunity is a critical aspect in the therapeutic interventions against certain infectious diseases. For example, the use of steroidal anti-inflammatory drugs in conjunction with antibiotics has been recommended for the treatment of *Pneumocystis* pneumonia in AIDS patients (48), and it has been demonstrated that treatment with anti-inflammatory drugs can improve the clinical condition of patients infected with *Mycobacterium leprae* (49). In some forms of leishmaniasis, uncontrolled inflammation of cutaneous and mucocutaneous tissues results in tissue destruction and disfigurement. Postkala azar dermal leishmaniasis and mucocutaneous leishmaniasis are pathological states associated with *Leishmania* infection, in which the development of disfiguring lesions, associated with low numbers of parasites, is one of the major aspects of morbidity (50–52). Consistent with these observations are the findings that $CD4^+$ T cells not only protect mice, but under certain circumstances can exacerbate lesion development (53, 54). Thus, there appears to be a balance between the number of effector cells required for protection and the pathology they can mediate. The data presented in this work suggest that manipulation of the endothelial selectins may provide a novel strategy to modulate the pathology associated with infection without affecting host immunity. In fact, there have been previous studies showing that interfering with selectins decreased pathology in several models of cutaneous inflammation (55–57). These data suggest that the molecular aspects of T cell migration to tissues during *Leishmania* infection could be exploited during the therapeutic treatment of infection-induced pathology.

Our data indicate that although P- and E-selectins facilitate T cell migration to the site of *L. major* infection, some T cells can still migrate to infected tissues and mediate protection in their absence. However, in other situations, P- and E-selectins are required for tissue-specific $CD4^+$ T cell migration (16, 18, 20, 21, 34–37). Why P- and E-selectins are sometimes required may be due to how rapidly T cells are needed in the tissues. Thus, in an acute infection, there may be greater reliance on P- and E-selectins than during leishmaniasis, an infection that develops over weeks rather than hours or days. It appears that in the absence of the endothelial selectins, there is inefficient, yet sufficient, T cell migration to *L. major*-infected tissues, resulting in smaller inflammatory lesions without affecting immunity. At present, we do not know how these T cells get to the site of infection. However, there are several reports demonstrating a role for other molecular interactions, such as VLA-4/VCAM-1 (58), LFA-1/ICAM-1 (59), and CD44/hyaluronan (60), in mediating lymphocyte rolling on inflamed endothelium, and these molecules have been implicated in the pathogenesis of several disease states (61–63). Blockade of one or all of these interactions in the presence or absence of the endothelial selectins may affect the development of inflammatory lesions and subsequently resistance to *L. major*.

In summary, these data demonstrate that the host-protective response during a cutaneous infectious challenge can develop and resolve in the absence of P- and E-selectins. However, the endothelial selectins contribute to the development of immunopathology associated with *L. major* infection. Based on the data presented in this work, we propose that modulation of selectin/selectin ligand interactions may be a novel therapeutic approach for treatment of the exaggerated infection-induced pathology that can sometimes be associated with leishmaniasis without compromising protective immunity.

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