[illegible]

M. La[illegible] Mb[illegible], G[illegible] K. D[illegible] K[illegible] a[illegible] R[illegible] c[illegible] a[illegible] d G. T[illegible]

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Levels of expression of costimulatory molecules have been proposed to influence the outcome of antigen-specific T cell priming. We found that ***Le*** [illegible] selectively modulated the expression of costimulatory molecules on various populations of epidermal cells. B7.2 expression was down-regulated on Thy1.2^{+} epidermal cells (keratinocytes) from disease-resistant C3H mice, but not from disease-susceptible BALB/c mice. In addition, epidermal cells from BALB/c mice showed a down-regulation of B7.1 expression on NLDC 145^{+} Langerhans cells. ***I[illegible] v*** [illegible] T cell priming experiments, using syngeneic epidermal cells as antigen-presenting cells (APC), showed that the production of IFN-[illegible] was inhibited when either B7.1 or B7.2 signaling pathways were blocked. Blockade of B7.2, but not B7.1, significantly inhibited the ability of epidermal cells to induce IL-4 production from CD4^{+} T cells. In addition, C3H CD4^{+} T cells, which were unable to secrete detectable levels of IL-4 in cultures with syngeneic APC, were now able to secrete IL-4 following presentation of ***L. maj*** [illegible] antigens by congenic BALB/K epidermal cells. Conversely, C3H epidermal cells supported the priming of BALB/K CD4^{+} T cells for IL-4 production [illegible]. Thus, the differential expression of B7 molecules on epidermal cells may not represent the sole factor governing the polarization of ***L. maj*** [illegible]-specific CD4^{+} T cells [illegible].

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K[illegible] d[illegible]: Rodent / Dendritic cell / Costimulatory molecule / Protozoan parasite

1 [illegible]

The protozoan parasite ***Le*** [illegible] induces cutaneous leishmaniasis. It exists as a flagellated promastigote in its insect vector, the sand fly. The vertebrate host becomes infected with ***L. maj*** [illegible] when the sand fly probes into the skin for a blood meal and injects parasites. Promastigotes are taken up by phagocytic cells, and within these cells

[I 21025]

Abb[illegible]: **LC:** Langerhans cells **L[illegible]:** ***Le*** [illegible] ***maj*** [illegible] **MFI:** Mean fluorescence intensity

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Because the skin represents the primary target organ of *L. major*, epidermal APC, such as Langerhans cells (LC), are likely to play a major role in the initiation of an immune response against *L. major*. In fact, previous studies have shown that epidermal LC, but not keratinocytes, present *L. major* antigens to T cells [28–30]. Fur-

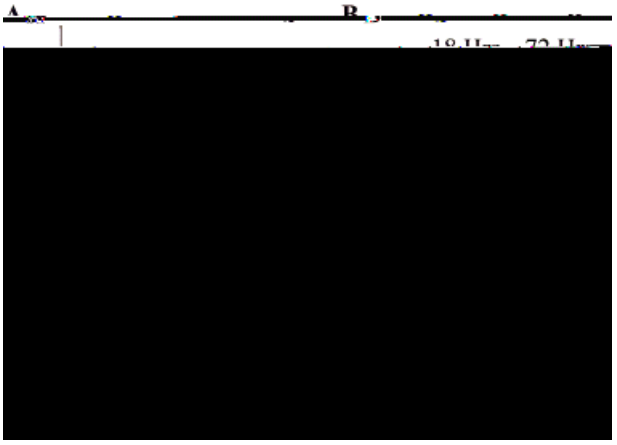
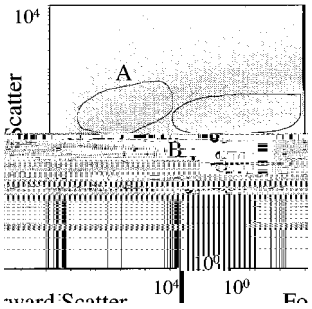
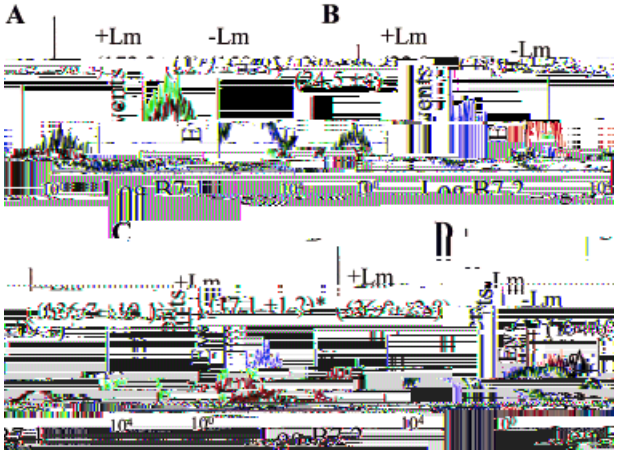


Figure Up-regulation of B7 molecules on epidermal cells cultured in the absence of *L. major*. Epidermal cells from (A, C) BALB/c and (B, D) C3H mice were analyzed for B7.1 using PE-conjugated anti-B7.1 and B7.2 expression using FITC-conjugated anti-B7.2 at 18 h (blue lines) and 72 h (black lines). Red lines represent isotype control Ab. For clarity, only isotype control Ab using cells cultured without *L. major* are shown. Similar results were obtained when epidermal cells cultured with *L. major* were stained with the same isotype control Ab. The data are representative of three experiments.

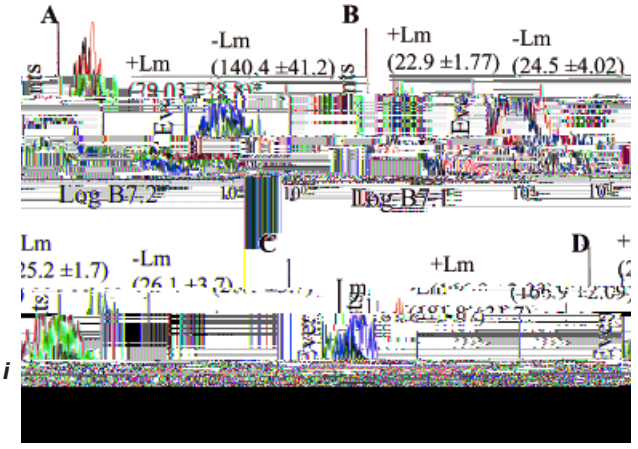


F Light scatter gates of NLDC 145 and Thy1.2$^+$ BALB/c epidermal cells. Epidermal cells were double-stained with NLDC-145 and anti-Thy1.2 mAb. The gates shown enclose NLDC 145$^+$ (A) and Thy1.2$^+$ (B) epidermal cells. No NLDC 145$^+$/Thy1.2 double-positive cells were identified. The light scatter gates shown here were used in subsequent experiments to enclose populations containing NLDC 145$^+$ and Thy1.2$^+$ cells.

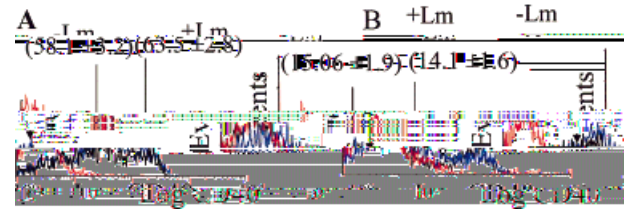
(light scatter gates of the different epidermal cell populations are shown in Fig. 2). Both B7.1 and B7.2 were equally up-regulated on NLDC 145$^+$ epidermal cells (LC) from C3H mice in the presence or absence of ***L. maj*** (Fig. 3A, B; ***p*** values in each figure were greater than 0.2). However, ***L. maj*** induced a selective down-regulation of B7.1 expression on BALB/c NLDC 145$^+$ cells [Fig. 4A; mean fluorescence intensity (MFI) of 140±41.2 in the absence of ***L. maj*** compared to 79±28.8 in the presence of ***L. maj***, $p=0.05$]. In contrast, B7.2 expression on BALB/c NLDC 145$^+$ was not affected in cultures containing ***L. maj*** (Fig. 4B). Our results also showed that ***L. maj*** differentially regulates the expression of B7.1 and B7.2 on Thy1.2$^+$ epidermal cells (***e.g.*** keratinocytes and dendritic epidermal T cells). B7.2 expression on Thy-1.2$^+$ C3H epidermal cells was down-regulated in the presence of ***L. maj*** (Fig. 3D; MFI of 36±9±2.87 in the absence of ***L. maj*** compared to a MFI of 17.1±1.2 in the presence of ***L. maj***, $p=0.03$). ***L. maj*** did not alter



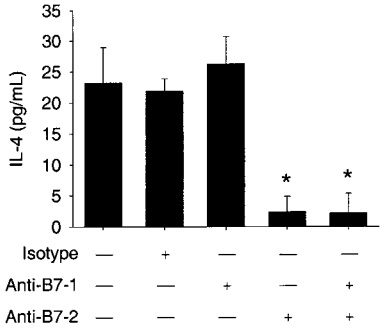
F ***L. maj*** selectively down-regulates B7.2 expression on Thy1.2$^+$ epidermal cells from C3H mice. Epidermal cells were isolated and cultured with (black lines) or without (blue lines) ***L. maj*** (Lm) promastigotes and analyzed by flow cytometry 3 days later by gating on (A, B) NLDC 145$^+$ or (C, D) Thy-1.2$^+$ C3H epidermal cells predetermined by light scatter. Red lines represent isotype control Ab. Numbers in parentheses indicate MFI ± SD from three experiments. The data are representative of three experiments. *$p=0.03$.



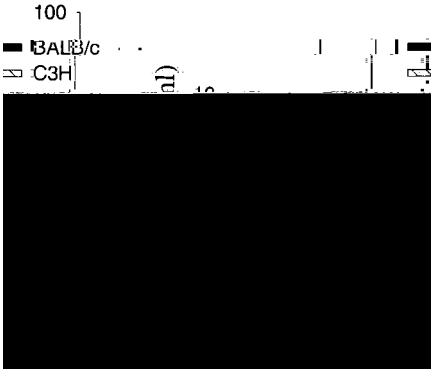
F ***L. maj*** selectively down-regulates B7–1 expression on BALB/c NLDC 145$^+$ LC. Epidermal cells were isolated and cultured with (black lines) or without (blue lines) ***L. maj*** (Lm) promastigotes and analyzed by flow cytometry 3 days later by gating on (A, B) NLDC 145$^+$ or (C, D) Thy-1.2$^+$ BALB/c epidermal cells predetermined by light scatter. Red lines represent isotype control Ab. Numbers in parentheses indicate MFI ± SD from three experiments. The data are representative of three experiments. *$p=0.05$.



F Equal up-regulation of CD40 expression on NLDC 145$^+$ epidermal cells derived from BALB/c and C3H mice in the presence or absence of ***L. maj***. Epidermal cells derived from (A) C3H and (B) BALB/c mice were cultured in the presence (black lines) or absence (blue lines) of ***L. maj*** (Lm) and CD40 expression was analyzed 3 days later. Light scatter gates were predetermined to enclose NLDC 145$^+$ cells. Red lines represent isotype control Ab. Numbers in parentheses indicate intensity MFI ± SD from three experiments. The data are representative of three experiments.



F B7.2, but not B7.1, on BALB/c epidermal cells costi- ***i***
mulates for IL-4 production by ***L. maj*** -specific $CD4^+$ T cells ***r***
i



Tab 1. production of IL-4 using a congenic C3H-BALB/K mouse model[a)]

APC-T cells	IL-4 (pg/ml)				
	No Ab	Isotype	anti-B7.1	anti-B7.2	anti-B7.1 + anti-B7.2
C3H-C3H	0	0	0	0	0
C3H-BALB/K	42.6 ± 5.1	35.2 ± 3.4	45.8 ± 6.2	10.4 ± 2.2*	3.1 ± 0.75*
BALB/K-BALB/K	52.1 ± 3.8	55.7 ± 4.3	59 ± 6.8	8.6 ± 3.5*	2.4 ± 0.65*
BALB/K-C3H	69 ± 4.3	55 ± 7.4	58.8 ± 7.1	6.7 ± 1.2*	2.9 ± 0.32*

a) The cultures were set up as described in Fig. 6 and Sect. 4. The data represent means ± SD from three experiments. * $p < 0.05$.

Tab 2. production of IFN- using a congenic C3H-BALB/K mouse model[a)]

APC-T cells	IFN- (ng/ml)				
	No Ab	Isotype	anti-B7.1	anti-B7.2	anti-B7.1 + anti-B7.2
C3H-C3H	5.8 ± 1.3	4.1 ± 1.8	2.8 ± 0.5*	1.9 ± 0.78*	0.29 ± 0.1*
C3H-BALB/K	4.6 ± 2.3	5.3 ± 1.7	3.2 ± 1.1*	2.5 ± 1.05*	0.35 ± 0.2*
BALB/K-BALB/K	6.9 ± 1.7	6.3 ± 2.2	3.4 ± 1.4*	2.9 ± 1.5*	0.28 ± 0.17*
BALB/K-C3H	5.7 ± 2.3	6.1 ± 1.8	2.8 ± 0.8*	3.3 ± 1.9*	0.4 ± 0.18*

a) The cultures were set up as described in Fig. 7 and Sect. 4. The data represent means ± SD from three experiments. * $p < 0.05$.

2.4 BALB/K-C3H

To determine whether factors other than B7 expression on epidermal cells influence cytokine production by $CD4^+$ T cells, we used a congenic BALB/K (disease-susceptible)-C3H (disease-resistant) model to analyze IL-4 and IFN- production . As shown in Table 1, epidermal cells from C3H mice were able to prime BALB/K $CD4^+$ T cells for IL-4 production. Conversely, BALB/K epidermal cells supported the production of IL-4 by $CD4^+$ T cells from C3H cells. In all cases, anti-B7.2, but not anti-B7.1 mAb, significantly inhibited the generation of IL-4 . These results suggest that costimulation for IL-4 production by $CD4^+$ T cells may be regulated by more than costimulatory molecules on epidermal cells since BALB/K T cells produced IL-4 when activated by either BALB/K T cells or C3H epidermal cells.

The production of IFN- was also assessed in the congenic model described above. Both C3H and BALB/K epidermal cells were able to prime $CD4^+$ T cells from BALB/K and C3H mice, respectively (Table 2). Consistent with the data reported in the syngeneic model (Fig. 7), the generation of IFN- was dependent on both B7.1 and B7.2 molecules, because addition of anti-B7.1 or anti-B7.2 to the cultures significantly inhibited the secretion of IFN- (Table 2).

3

The T cell cytokine profile elicited in the host represents a crucial factor in determining disease outcome in mice infected with *L. maj* [2–5]. During primary T cell responses, the pattern of cytokines elicited by Ag-specific T cells may be regulated, at least in part, by the strength and affinity of the interaction between accessory molecules and their coreceptors on T cells [19, 20, 22]. Therefore, the levels of expression of accessory molecules on APC able to initiate a primary immune response could represent a crucial factor in determining the outcome of T cell priming. Because the skin repre-

Table 3). On BALB/c epidermal cells, B7.2 expression was equally up-regulated on Thy-1.2$^+$ and NLDC-145$^+$ cells in the presence or absence of *L. major*, whereas B7.1 expression was down-regulated on NLDC 145$^+$ cells (Fig. 4A). Furthermore, the B7.2 signaling pathway on BALB/c epidermal cells appeared to be involved in IL-4 production by *L. major*-specific CD4$^+$ T cells, because addition of neutralizing anti-B7.2, but not anti-B7.1, mAb significantly reduced the levels of IL-4 produced (Fig. 6). These results suggest that costimulation via B7.2 on LC could promote the production of IL-4 in susceptible mice infected with *L. major*. This hypothesis is supported by previous findings showing that treatment of BALB/c mice with neutralizing anti-B7.2 mAb dramatically reduced the levels of IL-4 produced in the lymph nodes draining leishmanial lesions, and enhanced resistance to *L. major* infection [24]. Likewise, Corry et al. [23] reported that treatment of BALB/c mice with CTLA4Ig within the first week of infection completely abrogated progressive disease, suggesting that the priming of Th2

strated in studies showing that CD40-deficient mice on a resistant background were unable to control infection with *L. major* [25, 26]. Thus, we compared levels of CD40 expression on BALB/c and C3H epidermal cells. Both strains of mice showed up-regulation of CD40 expression on NLDC 145^+ cells to equal maximum levels in the presence or absence of *L. major*. However, a broader range of CD40 expression was observed on C3H cells compared to BALB/c cells (Fig. 5). It is unclear how this difference in the population of $CD40^+$ epidermal cells impacts the outcome of T cell priming, but further study to examine this issue is warranted.

The selective down-regulation of B7.2 on Thy-1.2^+ positive C3H epidermal cells (Fig. 3D) argues for potential important roles played by Thy-1.2^+ epidermal cells, such as keratinocytes, in cutaneous leishmaniasis. Since

4.4 *In vitro* culture of epidermal cells

Freshly isolated LC-enriched epidermal cells were resuspended in DMEM (Gibco BRL) supplemented with 10% heat-inactivated FBS (Hyclone, Logan, UT), 0.1 mM nonessential amino acids (Gibco), 0.05 mM 2-mercaptoethanol, 10 mM Hepes (Gibco), 1% penicillin-streptomycin, 50 μg/ml gentamycin (Sigma, St. Louis, MO) and aliquoted into 96-well round-bottom plates (2×10^4 cells/well in a total volume of 100 μl) in the presence or absence of *L. major* promastigotes at a ratio 2:1 (*L. major* : epidermal cell) and incubated at 37°C for 3 days. The infection rate of epidermal cells was determined by incubating LC-enriched epidermal cells with a mixture of acridine orange (5 μg/ml) and ethidium bromide (50 μg/ml) as previously described [30].

Syngeneic splenic $CD4^+$ T cells were added to epidermal cell cultures (2×10^5) to a final volume of 200 μl/well, and incubation was continued for another 5 days. $CD4^+$ T cells were obtained from spleen cell populations by negative selection. Cells were incubated with a cocktail of mAb (J11d, B220, H35–17, M5/114, 33D1) followed by anti-rat IgG-coupled magnetic beads, and then passed through MiniMACS columns (Miltenyi Biotec, Auburn, CA). $CD4^+$ T cells were routinely enriched up to 94% as determined by flow cytometry. The enriched $CD4^+$ populations were not contaminated by APC because they did not express MHC class II, as determined by flow cytometric analysis (data not shown).

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