Supporting information

Methods

Infection with *Leishmania major*

Mice were injected in the left hind footpad with $1X10^6$ promastigotes of *Leishmania major* MHOM/S U/73-5-ASKH strain (*L. major*). The thickness of the infected and contralateral uninfected footpad was measured as described (1).

To measure the immune responses to pathogens, draining popliteal lymph node cells ($3\text{-}10X10^4$) were cultured in the presence of *L. major* antigen for 48 h as described (1).

(1) Tominaga N, Ohkusu-Tsukada K, Udono H, Abe R, Matsuyama T, Yui K (2003) Development of Th1 and not Th2 immune responses in mice lacking IFN-regulatory factor-4. *Int Immunol* 15: 1-10.

Figure 6

IRF-4$^{-/-}$ mice are resistant to *L. major* infection

Mice were injected in the left hind footpad with 1X10^6 promastigotes of *Leishmania major* MHOM/S U/73-5-ASKH strain (*L. major*). The thickness of the infected and contralateral uninfected footpad was measured as described (1). (A) BALB/c (open circle, n=8), B6 (open triangle, n=4) and IRF-4$^{-/-}$ mice (closed circle, n=14) were infected with *L. major* promastigotes, and the increase in footpad thickness (%) was determined. The data represent mean ± SD of each group. (B) Four weeks after infection, 1x10^5 (gray bar) and 3x10^4 (open bar) draining popliteal lymph node cells were cultured for 48 h in the presence of *L. major* antigen. The levels of IFN-γ in the supernatant were determined by ELISA. Representative results of three independent experiments are shown. *: not detectable.

Figure 7

$CD4^+$ NK T cells from IRF-$4^{-/-}$ mice produce IL-4 in response to alpha-GalCer

(1) Splenic $CD4^+$ T cells were stained with FITC-anti-CD4 and with biotin-anti-DX5 mAb plus PE-streptavidin, and analyzed using FACS Aria. The proportion of cells within the square is indicated.

(2) Sorted $CD4^+$ $DX5^+$ cells ($5X10^4$) from BALB/c (open bar) and IRF-$4^{-/-}$ (dark bar) mice were cultured with spleic dendritic cells ($CD11c^+$ cells, $1x10^4$) in the presence (+) and absence (-) alpha-GalCer (200ng/ml) for 48h. The cytokine levels in the supernatant were determined by ELISA. The purity of $CD4^+DX5^+$ cells from BALB/c and IRF-$4^{-/-}$ mice was 93.3 and 70.6%, respectively. The lower purity of $CD4^+DX5^+$ cells from IRF-$4^{-/-}$ mice was due to the lower expression of DX5 marker and their small numbers.

Figure 8

RNA expression of Th2 cytokines by naïve $CD4^+$ T-cells.

Naïve ($CD62L^+$) $CD4^+$ T-cells from BALB/c (open circle) or IRF-$4^{-/-}$ (closed circle) mice were cultured with plates coated with anti-TCR mAb for 0-24 h. Messenger RNA levels were determined by real time PCR and were expressed as the ratio of DNA to G3PDH. Representative results of three independent experiments are shown.

Figure 9

The forced expression of IRF-4 in IRF-4$^{-/-}$CD4^{+} T-cells enhanced their Th2 cytokine production.

CD4^{+} T cells from IRF-4$^{-/-}$ mice were co-transfected with pcDNA3 or pcDNA3-mIRF-4 together with pmaxEGFP at the ratio of 5:1, cultured for 3h, and stimulated with plate-bound anti-TCR mAb (0-10microgram/ml) for 48 h. The proportion of cells expressing GFP reached ~40% 24 h after the gene transfer. Cell lysate was prepared from unstimulated cells, separated by 12.5% SDS-PAGE, blotted, and probed with anti-IRF-4 Ab. The blot was stripped, and re-probed with anti-actin Ab. The levels of IL-4 and IL-5 in the supernatant were determined by ELISA. The proportion of EGFP^{+} cells was 30-45%. Mann-Whitney's *U* test for unpaired observations was used to calculate *p* values. Representative results of three independent experiments are shown.

Figure 6.

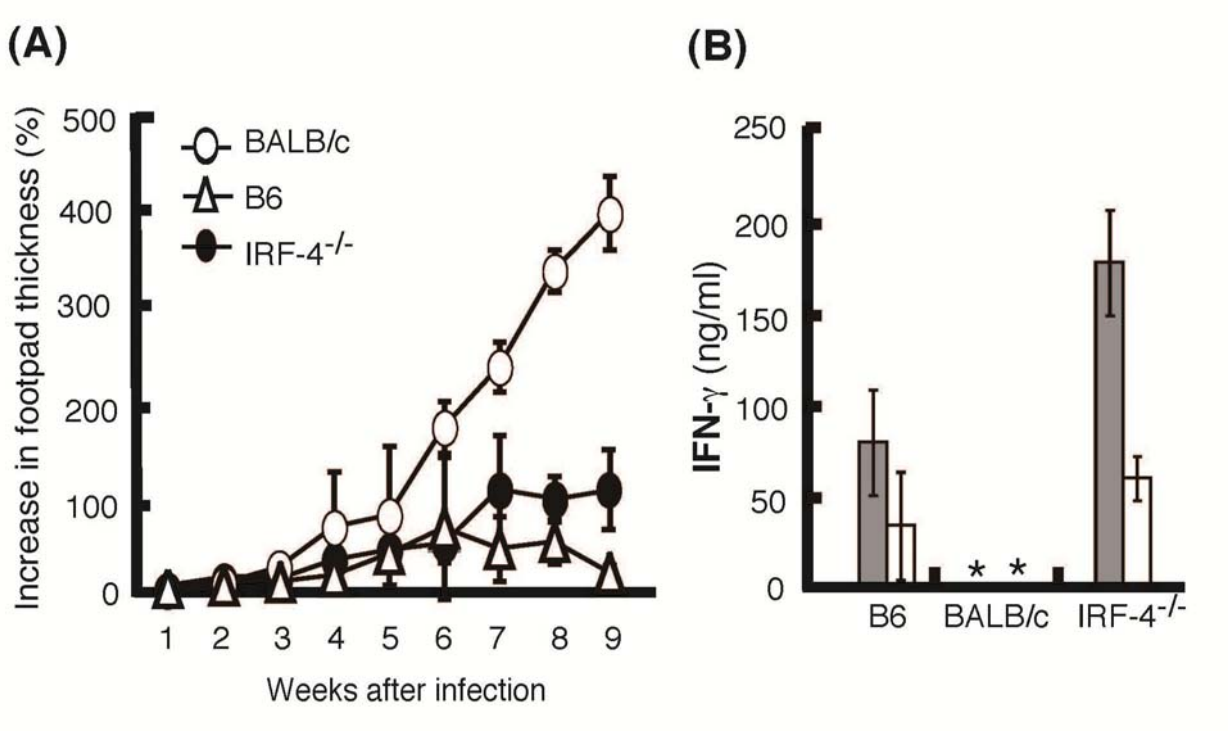
(A)
Increase in footpad thickness (%)
500
400
300
200
100
0
BALB/c
B6
IRF-4-/-
1 2 3 4 5 6 7 8 9
Weeks after infection
(B)
IFN-γ (ng/ml)
250
200
150
100
50
0
* *
B6
BALB/c
IRF-4-/-

Figure 7.

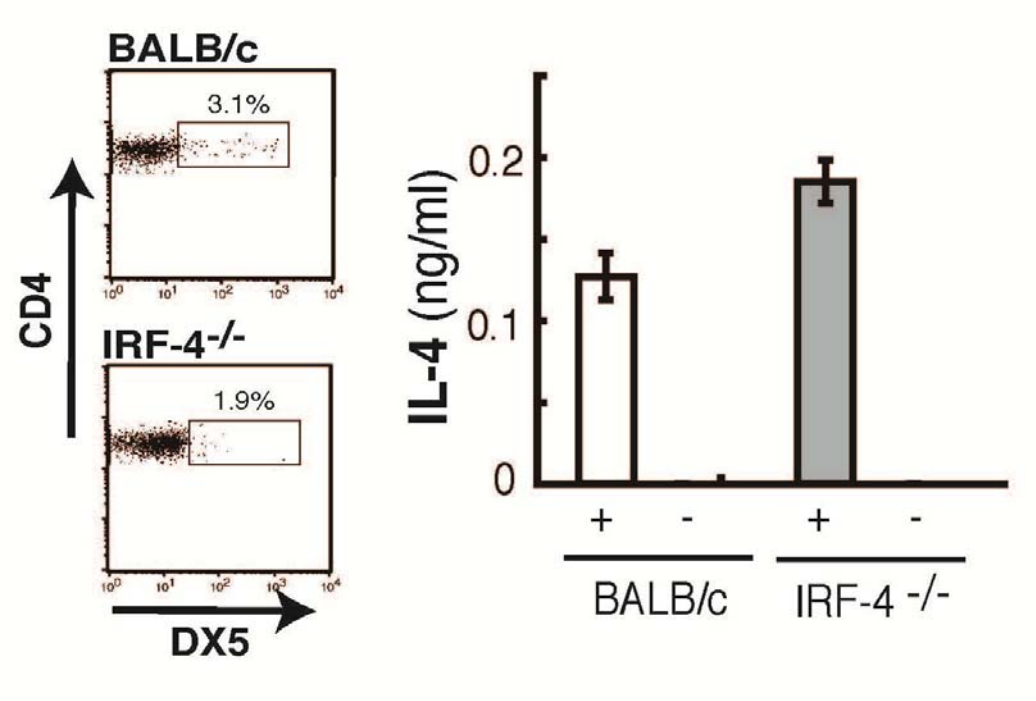

Figure 8.

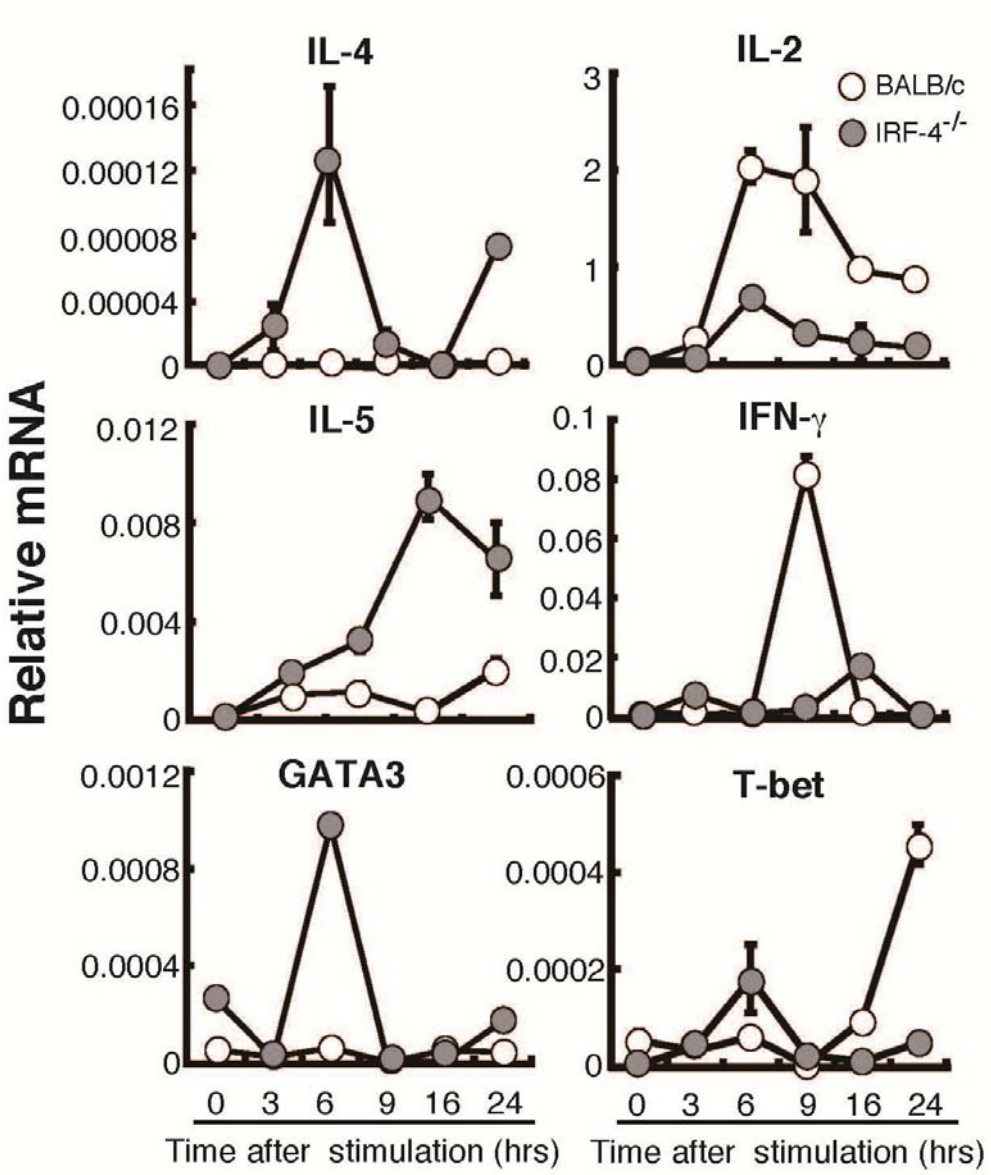
IL-4
IL-2
BALB/c
IRF-4-/-
IL-5
IFN-γ
GATA3
T-bet
Relative mRNA
0.00016
0.00012
0.00008
0.00004
0
3
2
1
0.012
0.008
0.004
0.1
0.08
0.06
0.04
0.02
0.0012
0.0008
0.0004
0.0006
0.0004
0.0002
0 3 6 9 16 24
Time after stimulation (hrs)
Time after stimulation (hrs)

Figure 9.

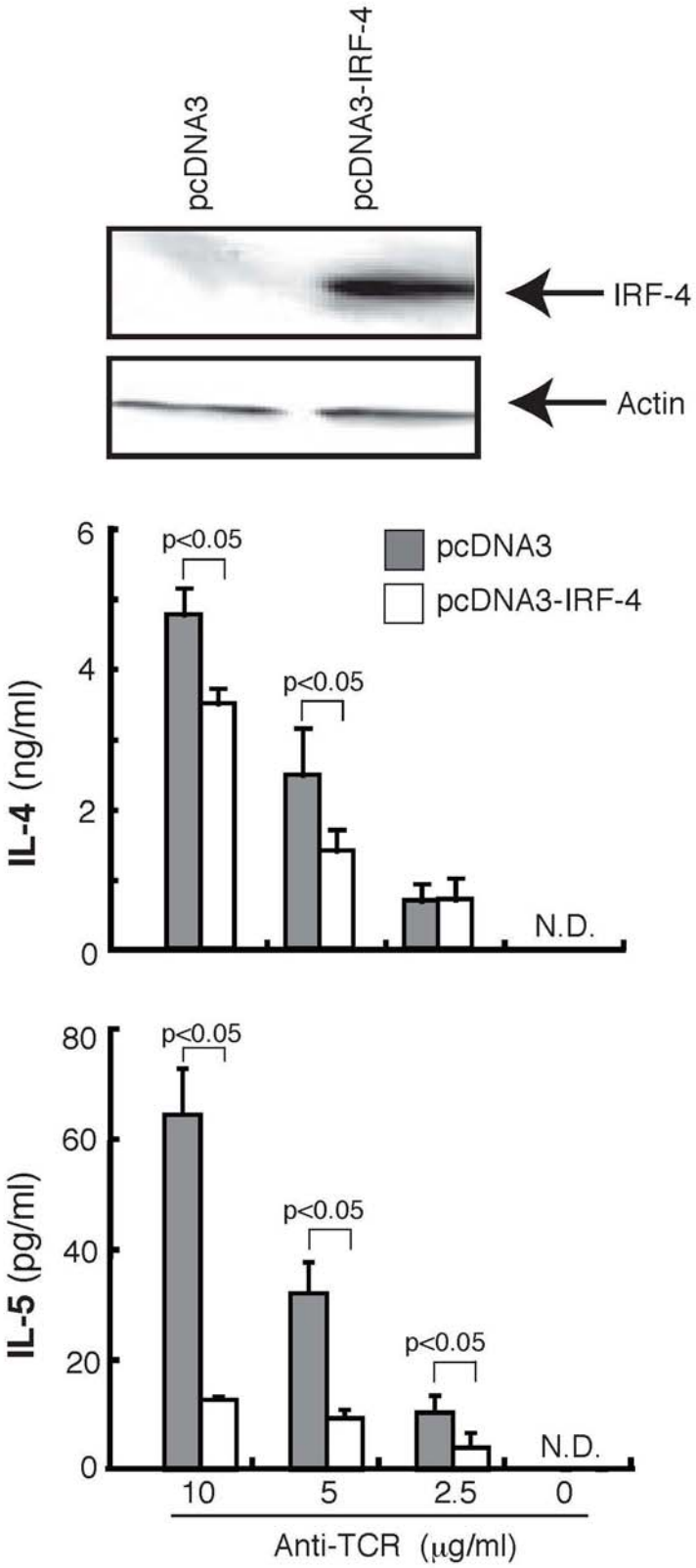
pcDNA3
pcDNA3-IRF-4
IRF-4
Actin
pcDNA3
pcDNA3-IRF-4
IL-4 (ng/ml)
p<0.05
p<0.05
N.D.
IL-5 (pg/ml)
p<0.05
p<0.05
p<0.05
N.D.
10
5
2.5
0
Anti-TCR (μg/ml)

Table S1 Primer sequences used for real-time RT-PCR

name	5’ primer	3’ primer
IL-4	TCATCGGCATTTTGAACGAG	TTTGGCACATCCATCTCCG
IL-5	CTCTCAGCTGTGTCTGGGCC	GCTTGTCAACAGAGCTCGGTG
IFN-gamma	GCATTCATGAGTATTGCCAAGTTT	GATTCCGGCAACAGCTGGT
IRF-1	ATTCCAACCAAATCCCAGGG	CTCCGGAACAGACAGGCATC