TD-IAI02: Immunity to Infection

(from Simona Stäger, James Alexander, Alun C Kirby, Marina Botto, Nico Van Rooijen, Deborah F Smith, Frank Brombacher & Paul M Kaye. *Nature Medicine* 9, 1287-1292)

I- Introduction

The importance of $CD8^+$ T cells in host protection against intracellular pathogens is well recognized, and $CD8^+$ T-cell priming is crucial for successful vaccination against leishmaniasis. Although genome sequencing projects now provide a plethora of potential vaccine candidates, few criteria exist for selecting from them, and antigen choice remains largely empirical. In addition, successful vaccination requires antigen targeting to the major histocompatibility class I processing pathway and a cytokine milieu that favours $CD8^+$ T-cell priming and differentiation.

Fc receptor (FcR)-mediated uptake of immune complexes facilitates class I–restricted antigen presentation, leading to the suggestion that vaccine efficacy might be improved by immunization with preformed immune complexes. Mice deficient in complement component C3 ($C3^{-/-}$) mount poor cytotoxic T-lymphocyte (CTL) responses to infection with influenza virus and lymphocytic choriomeningitis virus.

Natural antibodies are the germline-encoded IgM, IgG and IgA found in normal humans and other animals. They have low affinity compared with hypermutated antibodies generated during normal immune responses, and have broad reactivity to a variety of self-antigens. Most natural antibodies are produced by self-renewing B-1 B cells, which preferentially localize to the peritoneum. Natural antibodies serve as innate microbial recognition receptors, recognizing various bacterial cell wall components.

Hydrophilic acylated surface protein (HASP) B-1, a member of a family of proteins expressed by infective stages of *Leishmania* parasites, was recently identified by us as a lead candidate for vaccination against visceral leishmaniasis. Immunization with recombinant HASPB-1 induced antigen-specific $CD8^+$ T cells and long-term protection, which are both characteristics attributed to DNA vaccines.

II- Methods

Mice. We used the following mouse strains BALB/c, BALB/c-background IL-$4^{-/-}$ and IL-4 receptor □$^{-/-}$ (IL-4ra$^{-/-}$), SCID (Severe Combined Immuno Deficient); B6-background *C*1*qa*$^{-/-}$ (complement deficient), IL-$12^{-/-}$, and IL-4-GFP reporter (4get) mice.

Parasites. *L. donovani* amastigotes (strain LV9) were isolated from infected hamsters. Mice were infected with 2 10^7 amastigotes through the lateral tail vein. Hepatic and splenic parasite burdens were determined from Giemsa-stained tissue impression smears, and data were presented in Leishman Donovan units (LDU).

Antigen preparation and vaccination. Recombinant HASPB-1 was purified by reverse-phase high-performance liquid chromatography (97% purity; 0.17 endotoxin units per mg recombinant HASPB-1. Mice were vaccinated subcutaneously with recombinant HASPB-1 or ovalbumin and infected 3 weeks later.

III- Results

Figure 1.

Background: IL-4 deficient mice were immunized with recombinant HASPB-1 and challenged with Leishmania Donovani (**Figures 1a et 1b**). Spleen cells were isolated from vaccinated animals before challenge, restimulated with recombinant HASPB-1 and the frequency of IFN--producing cells was determined in BALB/c (**figure 1c**) and IL-$4^{-/-}$ (**figure 1d**)). HASPB-1-specific CTL activity was analysed in vitro (**figure 1e**). Protective capacity of CD8 T cells was assessed by adoptive transfer approach (**figure 1f et 1g**).

Fig.1a: Groups of control (●, ▲) and vaccinated (○, Δ) BALB/c (●, ○) and IL-$4^{-/-}$ (▲, Δ) mice (n = 5 per group) were challenged with *L. donovani*, and parasite burden (expressed in Leishman Donovan units) in the spleen was determined at the times indicated.

Fig.1b: Parasite burden of BALB/c mice (●,○) compared with IL-4ra$^{-/-}$ mice (▲, Δ), as in **a**. *;, $P < 0.05$; **, $P < 0.01$ compared with unvaccinated mice.

Fig.1c: BALB/c mice and Fig.1d: IL-$4^{-/-}$ mice were immunized with recombinant HASPB-1 (rHASBP-1; ●, ○) or ovalbumin (■, □), cultured with (●, ■) or without (○, □) recombinant HASPB-1 and assayed for IFN-ϒ-producing $CD8^{+}$ T cells. Horizontal bars indicate mean values. NS, not significant.

Fig.1e: CTL activity of BALB/c-derived (●, ○) and IL-$4^{-/-}$-derived (■, □) $CD8^{+}$ T cells against HASPB-1-expressing (●, ■) or control (○, □) targets.

Fig.1f: (spleen) and Fig.1g (liver): $CD8^{+}$ T cells from naive or HASPB-1 vaccinated BALB/c or IL-$4^{-/-}$ mice were transferred into BALB/c recipients, and parasite burden was determined on day 50 after infection with *Leishmania*.

Figure 1 (continued)

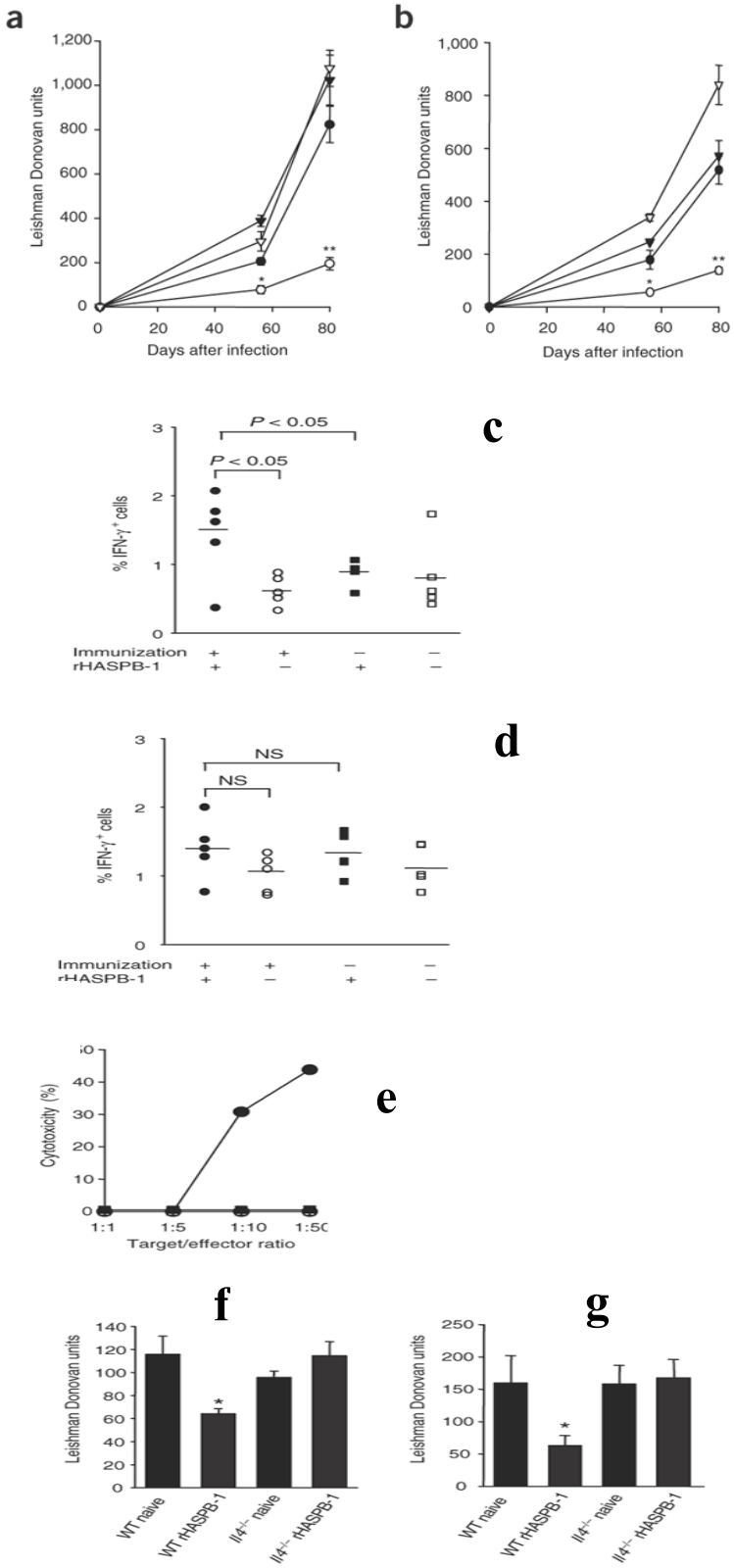
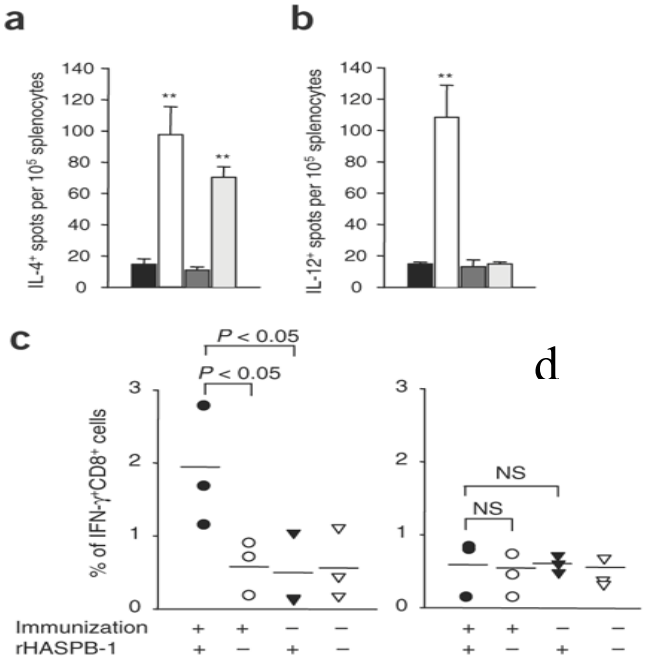


Figure 2

Background: Vaccination with recombinant HASPB-1 induced rapid IL-12p40 and IL-12p70 production.

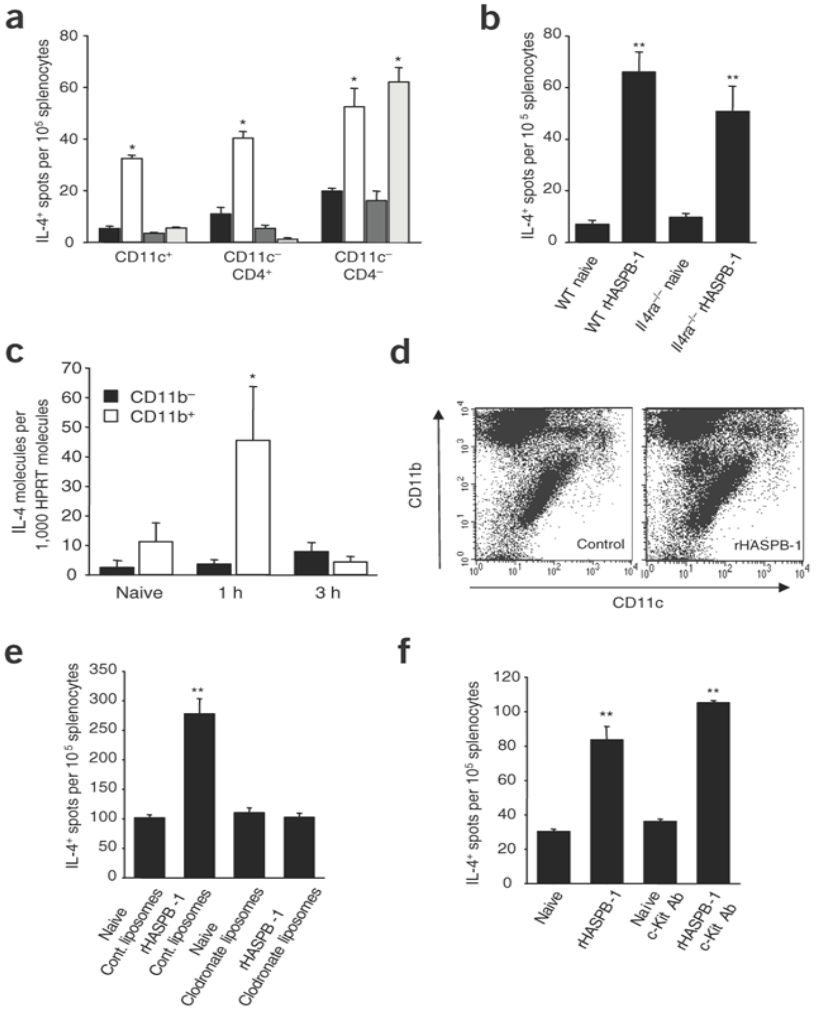
Fig. 2a: IL-4 and IL-12 Fig.2b: responses of spleen cells from BALB/c (■, □) and IL-4ra$^{-/-}$ (■, □) mice that were untreated (■, ■) or injected with recombinant HASPB-1 (rHASPB-1; □ , □). **, $P < 0.01$ compared with untreated mice. Fig. 2c: Spleen cells from individual C57BL/6 (left) and IL-12$^{-/-}$ (right) mice were immunized with recombinant HASPB-1 (●, ○ or ovalbumin (▲, Δ) were cultured with (●, ○) or without (●, Δ) recombinant HASPB-1 and assayed for IFN-γ-producing CD8^{+} T cells. NS, not significant.



Question 1

a) Comment results depicted on figure 1. Indicate the mediators and cells involved in this phenomenon.
b) Explain and discuss techniques used to obtain data represented by figures 1c, 1d or 1e.
c) Propose a schematic view of the cytokines network involved in vaccine-induced responses.

Figure 3



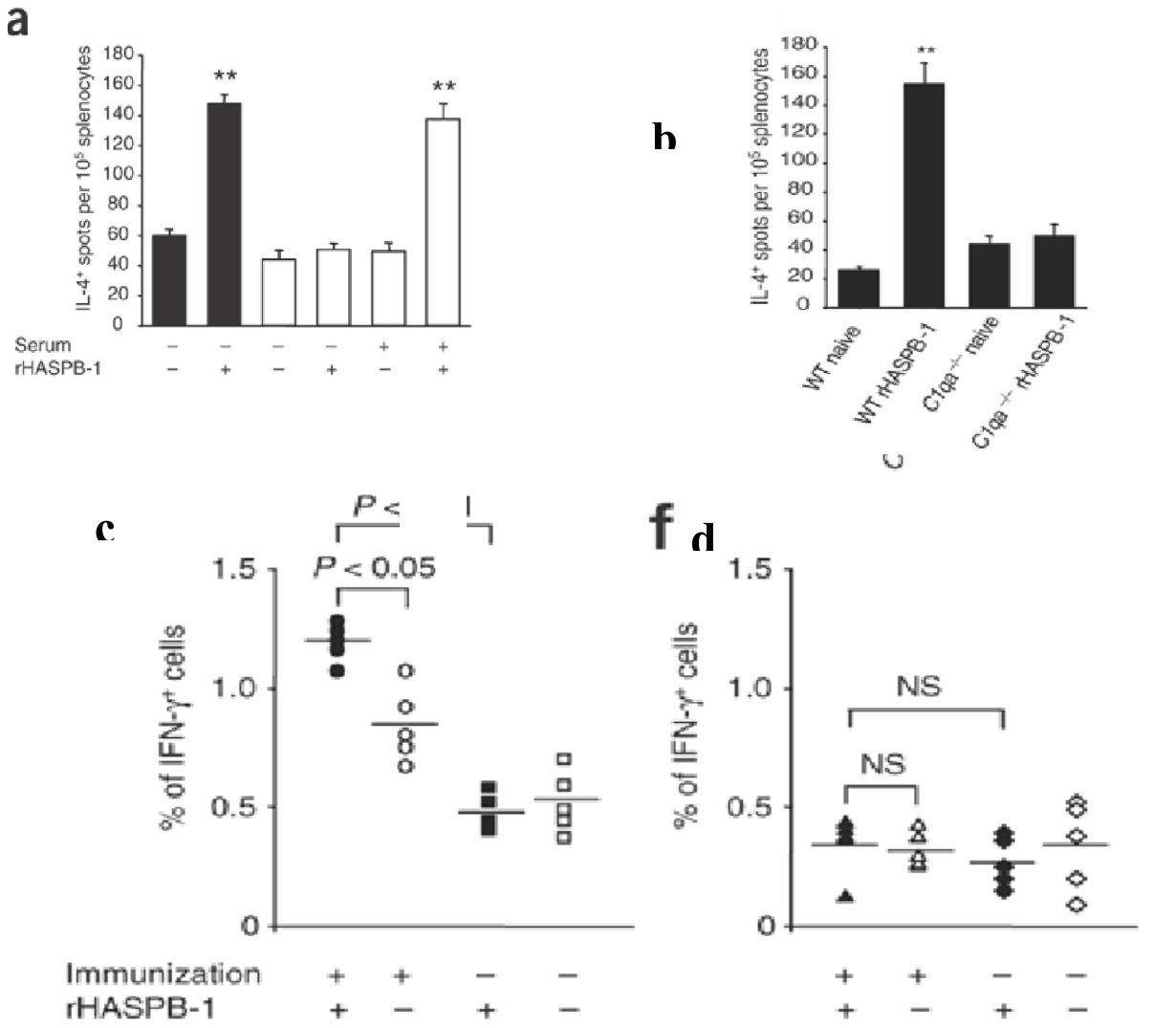
Background: 70-80% of Dendritic cells are $CD11c^{hi}$; 80% of $CD4^+$ T cells are $CD11c^-CD4^+$. Clodronate (dichloromethylene biphosphate) induces selective apoptosis in mononuclear phagocytes (monocytes, macrophages and dendritic cells). Monoclonal antibody directed to c-Kit depletes mast cells.

Fig.3a: Spleen cells were separated by magnetic beads and number of IL-4 producing cells were scored: BALB/c (■) and IL-$4ra^{-/-}$ (▣) mice, and BALB/c (□) and IL-$4ra^{-/-}$ (▣) mice injected with recombinant HASPB-1 (rHASPB-1).
Fig.3b: Cells were positively selected with CD11b were analysed.
Fig.3c: Spleen cells were recovered from naïve (■) or from HASPB-1-injected (□) mice. Cell were analysed by Real time PCR to determine IL-4 mRNA.
Fig.3d: IL-4 enhanced green fluorescent protein (EGFP) reporter "4get" mice were injected with recombinant HASPB-1 (right panel) or not (left panel). IL-4^+ cells were gated on FL-1 and then analyzed for CD11b and CD11c expression.
Fig.3e: Spleen cell from naïve or HASPB-1 injected BALB/c mice were treated with clodronate liposomes.
Fig.3f: Spleen cells from naive or HASPB-1-injected BALB/c mice c-Kit monoclonal antibody.

Question 2

What cells secrete IL-4 ? Explain.

Figure 4



Background : IL-4 secretion is absent in B-cell deficient mice and restored after serum transfer. SCID mice were injected with r HASPB-1 associated or not with sera from normal mice, as indicated.

Fig.4a: Number of IL-4 producing cells was determined from BALB/c (■) or SCID (□) mice.

Fig4b: Number of Il-4 producing cells was determined from BALB/c or *C1qa*$^{-/-}$ mice untreated or injected with rHASPB-1 as indicated.

Fig.4c: (wild type mice) and Fig.4d (*C1qa*$^{-/-}$ mice): Percent of IFNγ CD8+ producing cells was determined. Mice were injected with recombinant HASPB-1(●, ○, ▲, Δ) or Ovalbumin (■, □, ◆, ◊). Spleen cells were recovered and stimulated or not in vitro with recombinant HASPB-1, as indicated.

Question 3

Do T and B cells are involved in production of IL-4? Why? What key experiment allowed this demonstration?

Questions 4

Do and how immune complexes play a role in the IL-4 production ? Propose a title for this paper.