Th1-Th17 Cells Mediate Protective Adaptive Immunity against *Staphylococcus aureus* and *Candida albicans* Infection in Mice

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Abstract

We sought to define protective mechanisms of immunity to *Staphylococcus aureus* and *Candida albicans* bloodstream infections in mice immunized with the recombinant N-terminus of Als3p (rAls3p-N) vaccine plus aluminum hydroxide ($Al(OH)_3$) adjuvant, or adjuvant controls. Deficiency of IFN-γ but not IL-17A enhanced susceptibility of control mice to both infections. However, vaccine-induced protective immunity against both infections required CD4+ T-cell-derived IFN-γ and IL-17A, and functional phagocytic effectors. Vaccination primed Th1, Th17, and Th1/17 lymphocytes, which produced pro-inflammatory cytokines that enhanced phagocytic killing of both organisms. Vaccinated, infected mice had increased IFN-γ, IL-17, and KC, increased neutrophil influx, and decreased organism burden in tissues. In summary, rAls3p-N vaccination induced a Th1/Th17 response, resulting in recruitment and activation of phagocytes at sites of infection, and more effective clearance of *S. aureus* and *C. albicans* from tissues. Thus, vaccine-mediated adaptive immunity can protect against both infections by targeting microbes for destruction by innate effectors.

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Introduction

Staphylococcus aureus a d *Candida spp.* a e he ec d a d hi d eadi g cas f b d ea i fec i i h i a i ed a ie [1]. The e ga j i cas e a ea 150,000 c i ica b d ea i fec i s i g bi i f d a f hea h-ca e e e dis a d ~40,000 dea e ea i he US a e [1 4]. Ide ifica i f i s e echa f ec i e ada e i s i agai he e ga c i ica a he g s d f d e e f ac i e acci e a egi agai b h ga .

We e i s e ed ha acci a i i h he ec bi a N e i s f he ca dida A 3 adhe i (A 3 -N) i h a s i s h d ide (A (OH)$_3$) adj a i ed he s a f ice s e s e i fec ed i a e s i h e ha i c a f *Candida albicans* e hici i a *Staphylococcus aureus* (MRSA) [5 7]. The acci e e ai ed efficac agai b h i fec i i B ce deficie a i a bs T ce deficie a i a [6,7]. F he e, ad i e a fe f CD4+ T ce bs B220+ B ce i s e e s i ed he s a f eci ie ice i fec ed i h b h ga [6,7].

A h gh T ce a e ec a f A 3 -N acci e efficac , h c a e ca ab e f di ec i i g *C. albicans* *S. aureus* i c s e [8,9]. The ef e, he d ea effec f acci a i agai b h ga ha e e ai ed s c ea . I c h c , hag c i *C. albicans* a d *S. aureus in vitro* [8,10,11] a d *in vivo* [12 16], ecia he i ed i h -i f a a c i s ch a i e fe (IFN)-γ, hich d ced b CD4+ h c . The ef e, e h h i ed ha he e d effec f A 3 -N acci e- edia ed ec i agai b d ea i fec i cas ed b *S. aureus* a d *C. albicans* e e hag c i ed b -i f a a c i d ced b acci e- i e h c . We s gh e s cida e f da- e a e s i e e f ec i e h i s i b d ea i fec i cas ed b *S. aureus* a d *C. albicans*.

Results

CD4+ lymphocyte-derived IFN-γ was necessary for vaccine efficacy in mice infected with either organism

We e i s ab hed ha he A 3 -N acci e effec i e agai *C. albicans* i fec i i IFN-γ-deficie ice [6]. We s gh de e i e if IFN-γ a i ia e s i ed f acci e- edia ed ec i agai *S. aureus*, a d a de e i e if CD4+ T ce e e he e s i ed s ce f IFN-γ d c i edia e

s acci e efficac agai ¶ b h ga ¶. IFN-γ-deficie ice
hei i d- e, c ge ic c ¶ e es acci a ed i h A¶3 -N
s¶ A (OH)$_3$ & acci a ed) A (OH)$_3$ a e (c), a d b ¶ ed
a h ee ee ¶. T ee ¶ f i g he b ¶ , CD4+¶ e ic a d

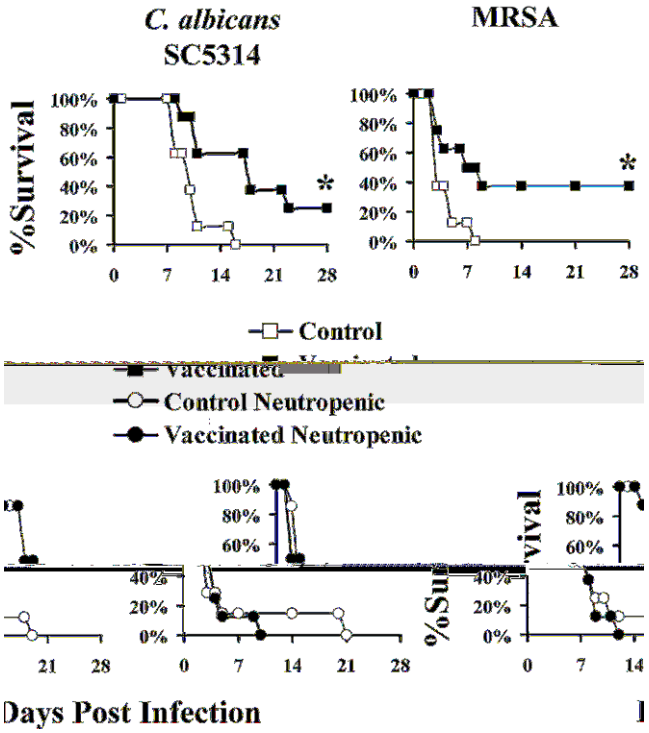

Figure 2. Chemotherapy-induced neutropenia ablated vaccine induced protection. Sixteen Balb/c mice per group were vaccinated with rAls3p-N plus $AlOH_3$ or $AlOH_3$ alone, and boosted three weeks later. Two weeks after the boost, half the mice were treated with cyclophosphamide. Two days later the mice were infected with *C. albicans* SC5314, 1.5×10^5, *C. albicans* 15563, 7×10^5, or MRSA LAC, 1.5×10^7. *p<0.05 for vaccinated vs. control by Log Rank test.
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d ice i ed he¶s a f id e eci ie ice, CD4+ T ce¶ f acci a ed, id- e d ice fai ed i e he¶s a f g $91^{phox-/-}$ eci ie ice (Fig. 3B).

CD4+ lymphocyte-derived IL-17A was also necessary for vaccine efficacy

The eed f d ¶ ea fs c i a hag c e¶ edia e acci e efficac ¶s gge¶ ed ha Th17 ce¶, hich a e ac b ec s i i g hag c e¶ he¶ i e¶ f i fec i [25,26], igh a a e. T de e i e he e os i e e f IL-17 a d Th17 ce¶ i edia i g acci e efficac , e acci a ed ice deficie i IL-17A, hei id e c ge ic c ice. IL-17A-deficie c ab ga ed acci e- edia ed efficac (Fig. 4A). Of e, i c a IFN-γ deficie c , IL-17A deficie c did e ace ba e he ¶s e i f i fec i i s acci a ed ice (c a i g¶s a f s acci a ed, deficie ¶. id e ice). T de e i e if CD4+ T ce¶ e e he i a ¶ s ce f IL-17A i edia i g acci e efficac , CD4+ T ce¶ f acci a ed c ice e e c ¶¶-ad i e a¶fe ed i eci ie ice (IL-17A-deficie d ce¶ a¶fe ed id e eci ie ice; id ed ce¶ a¶fe ed IL-17A-deficie eci ie ice). We a¶ e ea ed he¶s a i a¶s d i id e a d IL-17A deficie ice ha did s de g ad i e a¶fe ¶e a ¶i i e a d ega i e c ¶ f he ad i e a¶fe ¶ s d . Mice e e i fec ed he da af e ad i e a¶fe . O ce agai , he acci e i ed he¶s a i a f he ¶i i e c id e ice bs he ega i e c IL-17A deficie ice (Fig. 4B). Ad i e a¶fe f CD4+ ce¶ f acci a ed id e d

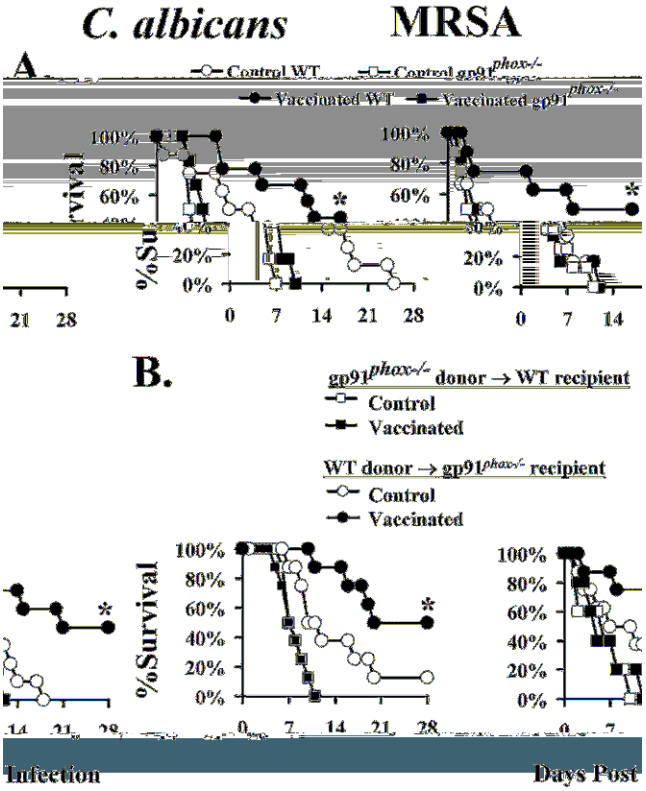

Figure 3. Phagocyte superoxide production is required for vaccine protection. N = 8 mice per group. Wild type C57BL/6 mice were infected with 1.5×10^5 *C. albicans* SC5314 or 2×10^7 *S. aureus* LAC; $gp91^{phox-/-}$ mice on a C57BL/6 background were infected with 1.5×10^3 *C. albicans* or 10^7 *S. aureus*. A) Mice were vaccinated and infected as above. *p<0.05 for wild type vaccinated vs. control by Log Rank test. B) CD4+ T cells, 5×10^6, from vaccinated or control, wild type or $gp91^{phox-/-}$ mice were cross-adoptively transferred iv to recipient mice 24 h prior to infection–wild type cells transferred to $gp91^{phox-/-}$ mice, and visa versa. *p<0.05 for wild type donor cells vaccinated vs. control by Log Rank test.
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ice i ed he¶s a f IL-17A-deficie eci ie ice (Fig. 4B). I c a , a¶fe f CD4+ T ce¶ f acci a ed IL-17A-deficie d ice id e eci ie ice fai ed i e ¶s a (Fig. 4C), c fi i g ha CD4+ T ce de i ed IL-17A a ec¶¶a edia e acci e efficac .

Vaccination induced Th1, Th17, and Th1/17 cells in mice

T defi e he s a i ¶ f ce¶ i ds ced b acci a i , ¶ ee¶ a d h de¶ e e ha ed f acci a ed a d c ice ee¶ f i g he b ¶. The ce¶ e e ¶ i s a ed *ex vivo* f 5 da ¶ i h A¶3 -N. A a ¶ f ¶s e a a ¶ c fi ed ha ce¶ f acci a ed ice ds ced ¶ig ifica e IFN-γ a d IL-17, a e a he es hi- ac i g che i e, KC a d MIP-1α, ha did ce¶ f c ice (Fig. 5A). IL-4 e e e de ec ab e i a ¶s e a a f c ce¶; e e e de ec ab e a e (< 2 g/) i ¶s e a a ¶ f 4 f he 8 ice i he acci a ed g s . H e, IL-10 a d IL-13 e e e highe i ¶s e a a ¶ f acci a ed ha c ice. Le e¶ f TGF-β a d IL-6 e e a d ¶ig ifica diffe e i ¶s e a a ¶ f acci a ed c ice. Ss e a a ¶ f ¶ i s a ed, i s ce¶ a ed e ha ced hag c ic i i g f *C. albicans* a d *S. aureus ex vivo*, c a ed ¶s e a a f c ce¶ (Fig. 5B).

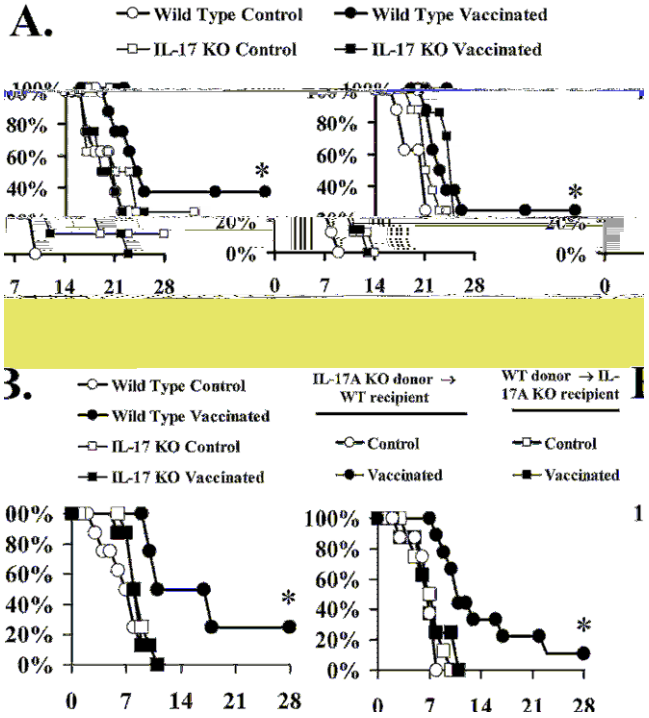

Figure 4. CD4+ T cell derived IL-17A was required for vaccine protection. A) Balb/c or IL-17A deficient mice on a Balb/c background (n = 8 per group) were vaccinated with rAls3p-N plus Alhydrogel or Alhydrogel alone, with a boost at 3 weeks. Two weeks after the boost, all mice were infected with 2.5×10^5 *C. albicans* SC5314 or 2×10^7 *S. aureus* LAC. B) Balb/c mice or IL-17A deficient mice on a Balb/c background, n = 8 per group, were vaccinated with rAls3p-N plus Alhydrogel or Alhydrogel alone. Two weeks after the boost, splenic and lymph node CD4+ T cells, 5×10^6, from vaccinated or control, wild type or IL-17A-deficient mice were cross-adoptively transferred iv to recipient mice, wild type donor to IL-17A deficient recipient, IL-17A donor to wild type recipient, 24 h prior to infection with *C. albicans* SC5314, 2.5×10^5 inoculum. *$p<0.05$ for wild type donor vaccinated vs. control by Log Rank test.
doi:10.1371/journal.ppat.1000703.g004

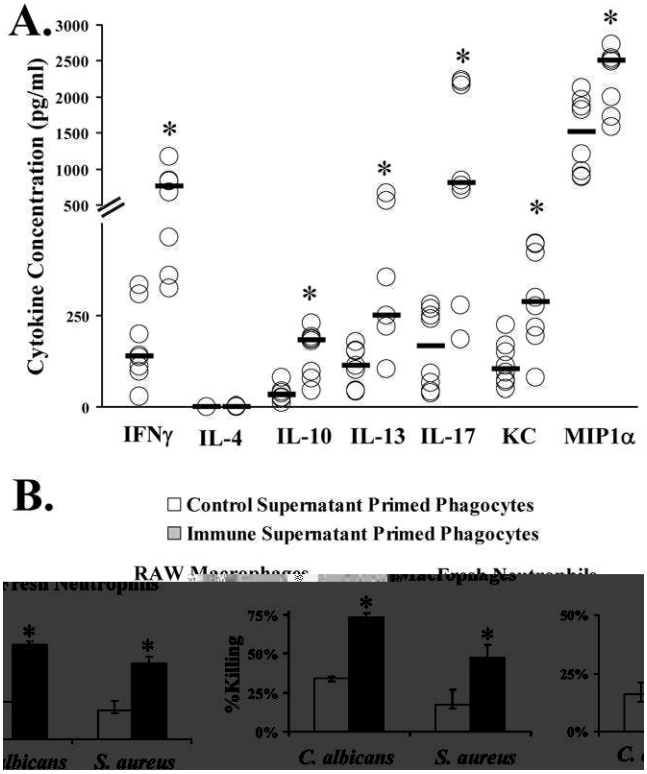

Figure 5. Vaccination primed lymphocytes to produce pro-inflammatory, Th1/Th17 cytokines which enhanced phagocytic killing of both organisms. A) Balb/c mice, n = 8 per group, were vaccinated with rAls3p-N plus Alhydrogel or Alhydrogel alone = Control. Two weeks after the boost splenocytes and cervical and axillary lymph node cells were harvested and incubated for 5 days with rAls3p-N. Supernatant was harvested for determination of cytokine levels. Median and interquartile ranges are shown. *$p<0.03$ vs. Control. B) RAW murine macrophage cells or freshly harvested murine neutrophils were primed with the above supernatants for four hours prior to incubation for one additional hour with *C. albicans* SC5314 (20:1 RAW to *C. albicans*; 10:1 neutrophils to *C. albicans*) or *S. aureus* LAC (5:1 RAW to *S. aureus*; 10:1 neutrophils to *S. aureus*). The culture wells were overlaid with agar and colonies counted the following day. Percent killing reflects reduction in colony forming units compared to number of organisms added to the wells. Median and interquartile ranges are graphed. *$p<0.05$ for immune vs. control supernatant.
doi:10.1371/journal.ppat.1000703.g005

I ace s a c ie a a ¶i f he ¶i s a ed ce ¶ de - ¶ a ed ha , acci a i e s ed i i c ea ed f eO s e ci e f Th1 (CD4+IFN-γ+), Th17 (CD4+IL-17+), a d Th1/17 (CD4+IFN-γ+IL-17+) ce ¶ i d ai i g h de h c e (Fig. 6 a d Fig. S2 a d S3) c a ed he f eO s e ci e i s , acci a ed ice. Ms i e CD4+CCR6- ce ¶ e e e iched f he Th1 he e, a d CD4+CCR6+ ce ¶ e e e iched f he Th17 he e. H e e, a s b a ia i f CCR6+ ¶ e c e, a d a ics a CD4+CCR6+ h c e, e e Th1/17 (IFNγ+IL-17+) ce ¶. The Th1/17 he e a ed i a f s d i CD4+CCR6+ ce ¶, i he CD4+CCR6- ce ¶.

Vaccination resulted in enhanced phagocyte recruitment and inflammatory cytokine production in the kidneys during *C. albicans* and *S. aureus* iv infection

T c fi he *in vivo* bi gica e a ce f he *ex vivo* h c e he e , acci a ed c ice e e i fec ed ia he ai ei ih *C. albicans* *S. aureus* 2 ee ¶ f ig he b ¶. A da 4 ¶ -i fec i (he da bef e c ice e e a ici a ed begi d i g), bs de fi fec i a d c ie e e ¶ i h ge a e f i di ids a a ed id e ¶ (ia age ga) e e de e i ed. Le e ¶ f e e ida e (MPO), hich i c ¶ is i e e ed a he ei e e i es hi ¶ a d ha bee e e ¶i e s ed i e is ¶ ¶ s die O s a if es hi if s i s e ds i g fec i a d if a ai [27 31], e ea ea s ed.

Vacci a i e s ed i a ~10-f d eds c i i id e f ga bs de a d ~5-f d eds c i i id e bac e ia bs de (Fig. 7A). MPO e e ¶ e e i ce a ed i , acci a ed ice ea i e c ice i fec ed i h ei he ga i (Fig. 7B). A ece ¶ s d e e d a 95% c e a i be ee ga f s ga bs de a d es hi if s i ice i fec ed i h diffe e ¶ a i ¶ f *C. albicans* *C. dubliniensis* [32]. The ef e, a e ha ced es hi if s e s i g f , acci a i c s d be ffe e b he di i ihed ¶ i s s ¶ f es hi if s cas ¶ ed b eds ced f s ga bs de i he , acci a ed ice. T i a e he i ac MPO e e ¶ f acci a i , a d ¶ e i fi fec i , e adj s ¶ ed al ¶ s e MPO e e ¶ i i di ids a a ed ga ¶ f he f s ga bac e ia bs de i h ¶ e i di ids a ga ¶. Vacci a i e s ed i a a ed ice ea e i es hi if s ea i e he i fec i s ¶ bs de f ga i i he s e (Fig. 7B). B h i a h g , he i fa a i fi a e a ed i a -

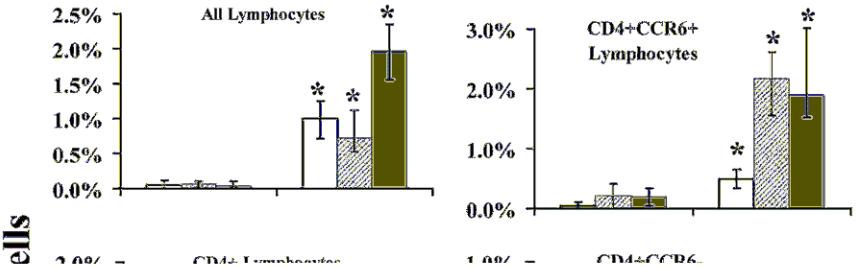

Figure 6. Vaccination primed Th1, Th17, and Th1/17 cells in draining lymph nodes. Balb/c mice, n = 4 per group, were vaccinated with rAls3p-N plus Alhydrogel or Alhydrogel alone = Control. Two weeks after the boost cervical and axillary lymph node cells were harvested and incubated for 5 days with rAls3p-N, followed by 6 h with PMA/ionomycin and monensin. Cells were fixed, permeabilized, and stained for CD4, CCR6, IFN-γ, or IL-17. Intracellular cytokine analysis was conducted by flow cytometry. "All Lymphocytes" were gated based on size by FSC and density by SSC; CD4+ and CCR6+ lymphocytes were further gated from the "All Lymphocyte" population by fluorescence signal indicating surface expression of these markers. Median and interquartile ranges are shown. *$P<0.05$ vs. Control.
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es hiic, ih¶ca e ed f ci f ac hage¶. Se i-OS a ia&i e ¶c ig f h¶ ah- g ¶ec i ¶ b abi ded ah- g¶ e i ae es hi if s i ¶s e ac c da ih he OS a ia&i e MPO e e¶.

C c da ih *ex vivo* c ie ea&s e e¶, ab- s e e¶ f IFN-γ, IL-17, a d he es hi-ac i g CXC che ie, KC, e e highe i he id e¶ f& acci a ed& e¶s¶ c - ice (<0.05 f a c a i ¶ f& acci a ed& ¶. c - e¶ f a hee c i e, i ice i fec ed ih *C. albicans* *S. aureus*). Af e adjs¶ i g f i fec i s¶ bs de i i di ids a ga¶, & acci a i a ed i ce ed c ie e¶ ea&e i fec i s¶ bs de (Fig. 7C).

H¶ ah- g c fi ed a a ed i ce e i ga¶ bs de i he& acci a ed ice& e¶s¶ c - ice i fec ed ih ei he ga¶ (Fig. 8). Ns e s¶ ic ab¶ce¶¶e ih h ha a d ¶es d h ha e e e ¶ e e¶ca e ed h s gh s he id e¶ f c - ice i fec ed ih *C. albicans*. Mic ab¶ce¶¶e e e a¶ f s di he id e¶ f& acci a ed ice, bs ¶ f he ab¶ce¶¶e had f s ga e e e &, i ibe, a d h¶ e fe ab¶ce¶¶e ih f s ga e e e ¶ c ai ed ba ¶ e ¶ a h ha fag e ¶. C - ice i fec ed ih *S. aureus* had a ge e a ab¶ce¶¶e ih s e s¶ g a ¶ai i e c ci G a ¶ai. Vacci a ed ice a¶ had e a ab¶ce¶¶e ih e e¶i e es hi if s, bs i ¶ ab¶ce¶¶e fe e ¶ a h- c cca ga i ¶ e e¶ee G a ¶ai i & acci a ed ha c - ice (Fig. 8).

Discussion

O e h he¶ ega di g he fais e da e de& e a effec i e& acci e agai ¶ *S. aureus* *Candida* ha bee he eed ¶i s a e s¶ di s s i e& i s e ce fac ¶ f ¶s ch c - e a h ge¶, he ea ¶ & acci e da e ha e a ge ed - e & i s e ce fac [33,34]. H & e, e ha e & i s¶ c fi ed ha hs a i s i i ei he ec¶¶a ¶s fficie f A¶3 -N & acci e-i ds ced ec i agai ¶ ei he ga i [6,7]. Fs he e, h g s¶ di s i f *ALS3* i *C. albicans* d e¶ e¶s i a- ¶¶ f a h ge ici *in vivo* i ice, ¶ he ec i ediaed b he A¶3 -N& acci e i he e¶s f ab ga i f A¶3 & i s e ce fs c i ¶. The c s e ¶s d c fi ¶ ha & acci a i ca be effec& e b a ge i g he ga i f de¶ s c i b i ce& i g he OS a i a d ic bi- cida fs c i ¶ f i a e hag c ic effec ¶ a he ¶ie f i fec i , i e ec& e f affec i g & i s e ce fs c i ¶ i he ga i . The ef e, e ia & acci e a ige ¶ eed be e ic ed ic bia & i s e ce fac ¶, a d ca be e a ded ic s de a a ge a ige hich e¶s ¶ i a e Th1 a d/ Th17 i s e e ¶e agai ¶ he ga i . The e da a a e c c da ih he e ab i hed - e f Th17 ce ¶ i ediaig ec i f- i g i s ia i f ice agai ¶ *Mycobacterium tuberculosis*, *Helicobacter pylori* a d *Pseudomonas aeruginosa* [31,35,36].

I s & acci a ed a i a ¶, deficie c i IFN-γ bs IL-17A e ace ba ed he¶& e i fi i fec i cas¶ ed b b h *S. aureus* a d *C. albicans*. The e e¶s ¶ a e c c da ih ece ¶ s die¶ de ¶ a i g ha IL-17-deficie ice e e e¶s¶ce ibe b d ea i fec i cas¶ ed b *S. aureus* [37] i & a& i e gai ic i fec i cas¶ ed b *C. albicans* [38]. Fs he e, a ece ¶ s d e ed ha ab ga i f he dec i -2 ece b c ed Th17 id s c i b *C. albicans* i ice, bs d¶ ie he ac f a Th17 e ¶ e did affec he abi i f ice c ea fs gs¶ f

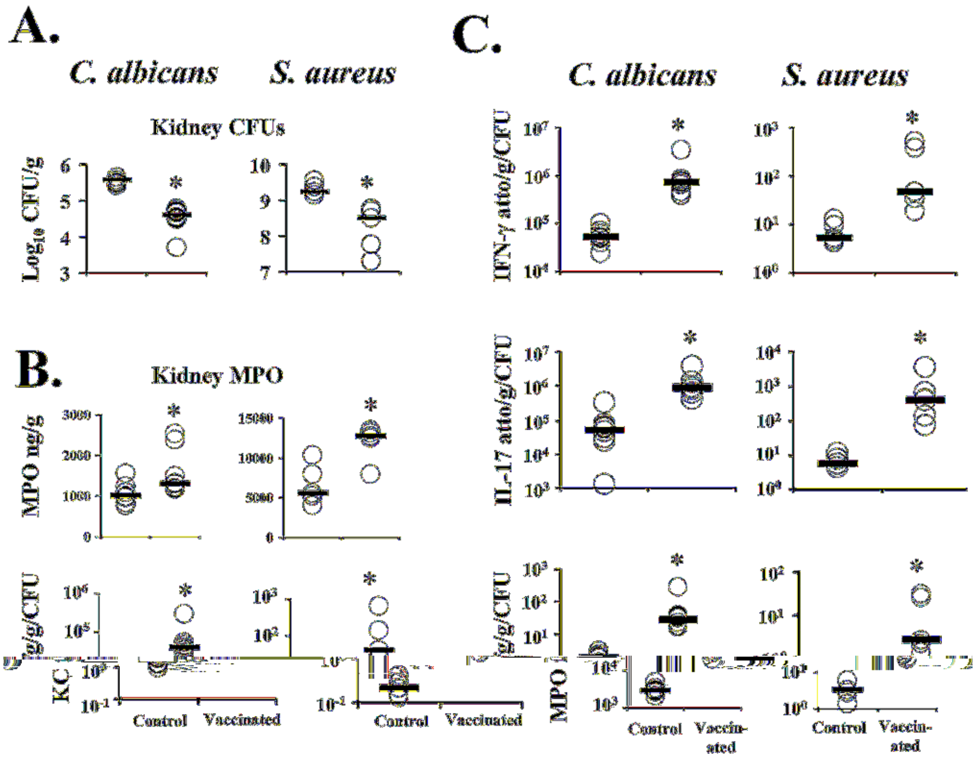

Figure 7. Vaccination reduced infectious burden and stimulated neutrophil influx by MPO and pro-inflammatory cytokine levels in kidneys. Balb/c mice, n = 8 per group, were vaccinated with rAls3p-N plus Alhydrogel or Alhydrogel alone. Two weeks after the boost, mice were infected iv with *C. albicans* SC5314, 2×10^5, or *S. aureus* LAC, 3×10^7. Four days after infection, individually marked kidneys (primary target organ of infection for both models) were harvested, homogenized, and quantitatively cultured (A). MPO levels (B) and cytokine levels (C) in organ homogenates were measured by ELISA. MPO levels are shown both as raw values in ng/g of kidney tissue, and also normalized to burden of infection in each individually marked organ in fg/g tissue/CFU per organ. Cytokine levels are shown normalized to organ CFU burden. Median and interquartile ranges are shown. *p<0.05 vs. Control by Mann Whitney U test.
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se ds i g e ic i fec i [39]. C ec e , he e s i dica e ha Th17 ce /IL-17A a e ec a f a s i e h defe e agai d e i a ed ca didia .

I c , IL-17 ha bee h be c i ica f h defe e agai cs a e s a d ha gea i fec i cas ed b *S. aureus* [37] a d *C. albicans* [40], ec e . Fs he e, s s a e d c da i h he e i s fi di g ha IL-17 ece -deficie c de a e e ace ba ed he e i f b ea i fec i cas ed b *C. albicans* [41]. The ac f a e i e e f IL-17A edia e a h defe e agai d e i a ed ca didia i e ef ec he abi i f IL-17F, hich a ac a he c IL-17 ece , c e e a IL-17A deficie c . Diffe e c i *C. albicans* i fec i g ai a d s e h ai a a acc s f diffe e c be ee s s d a d he i . H e e, a c i ica i ha IL-17F c s d c e a e f he e i e e f IL-17A i edia i g ec e, acci e- i cs ced, ada e i s i , i ce IL-17A deficie c ab ga ed acci e efficac .

We c fi ed ha he A 3 -N acci e ecifica i ed e ic a d h de h c ds ce high e e f b h IFN-γ a d IL-17, a e a he es hi che i , KC a d MIP-1α (he a e f hich i che ac ic f b h s c ea ce a d es hi [42 47]). The ed i a IFN-γ e i i h d a f s d i CCR6- Th1 ce hich did ds ce IL-17 (CD4[+]CCR6[−]IFN-γ[+]IL-17[−]), a d he ed i a IL-17 e i i h d a f s d i CCR6+ ce . H e e, e a f s d s a ia s be f Th1/17 ce , hich e e ceeded he f e c f Th17 ce ,

i he CD4+CCR6+ f ac i . The Th1/17 ce e e f s d i s a e cs e i he CCR6+ f ac i , a d e e f s d i he CD4+CCR6- f ac i . Rece s die ha e i dica ed ha ea a a ed ei i e Th17 ce ia ac a i f he a e ece [48], a d ha O-i ed a ei ca ac a e IFN-γ ds c i ia iga i f TLR4 [49]. Si ce he A 3 -N ei ha O-i ed ea high a e g s , c - iga i f he a e ece a d TLR4 a ige e i g ce a e ab e i ds c i f Th1, Th17, a d Th1/17 ce . The e f ecific a ige e i g ce i i i g h c f Th1, Th17, ds a Th1/17 cs e s de i iga i .

We f s a ia i i he a s be f s i g ice f e e i e e e i e , a gi g f a high a 87% a 12.5%. Va ia i i s c e a e i e acc s ed f b a ia i i i fec i s i cs s a d i fec i g ai . Os cha e ge de, s i g he a da d SC5314 c i ica a e f *C. albicans*, e e e ig s , a d c ide ab e ig s ha cha e ge i h he c i ica ai f *C. albicans* [6,50,51], ide ced b he s e i efficac ee i he cs e s d i h a he c i ica b ea a e f *C. albicans* (15563). We ha e e i s h ha ice i fec ed i h he i cs a f SC5314 s ed i h e e e i e die f e he i g e ic h c [52]. Ca dida e ic h c cas >50% ai i hs a d ie ea e i h a ifs ga he a [4]. He ce, achie e e f s a a achi g 50% b acci a i a e fe ef ec ea i gfs ec i . Fs he e, he e e i e i hich 12.5% s a a ee i he acci a ed a a a ad e

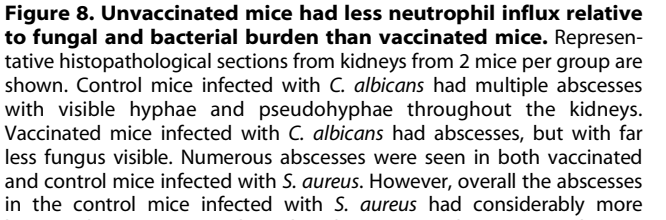

Figure 8. Unvaccinated mice had less neutrophil influx relative to fungal and bacterial burden than vaccinated mice. Representative histopathological sections from kidneys from 2 mice per group are shown. Control mice infected with *C. albicans* had multiple abscesses with visible hyphae and pseudohyphae throughout the kidneys. Vaccinated mice infected with *C. albicans* had abscesses, but with far less fungus visible. Numerous abscesses were seen in both vaccinated and control mice infected with *S. aureus*. However, overall the abscesses in the control mice infected with *S. aureus* had considerably more bacteria by gram stain than the abscesses in the vaccinated mice. Sections were stained by PAS (for *C. albicans*) or H&E (to show the neutrophil influx and the extent of tissue necrosis) and Gram stain (to show *S. aureus* dark purple clusters). Magnification = 1000×.
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a¶fe e ei e , i hich i s ece¶ f id e ice e e a¶fe ed i IL-17A-/- eci ie ice. Ths¶, hi e IL-17A ds c i f CD4+ i s e T ce¶ ca a¶fe eci , ds c i f IL-17A b he ce e a be e$_O$s ied achis e a i a eci . S ecifica , e s i-s¶ f s d ha i s e CD8+ T ce¶ c s d a¶fe eci agai¶ *S. aureus* [7], a d ac hage¶ de d i ic ce¶ ca ds ce -i fa a c i e¶ sch a IFN-γ, ¶ s gge¶ i g ha he¶e ce e a a a adjs c i e -e a d be e$_O$s ied f fs acci e- ediaed eci .

We s i s¶ e ed ha c c h¶ ha ide-i ds ced es-e ia did c e e ab ga e acci e-i ds ced eci ds i g¶ s h e$_O$s e di¶e i a ed ca didia [53]. I c , i he cs e ¶ s d , e did fi d a ab ga i f eci agai¶ b h ca dida¶ ai¶ a d agai¶ *S. aureus*. The i ¶ s d s¶ ed a diffe e bs e a ed acci e i s ge , A¶1 -N, i ¶ ead f A¶3 -N. A e , he i ¶ s d s¶ ed C e e F es d¶ Adjs a (CFA), A(OH)$_3$. The g ea e efficac f he f e adjs a a acc s f he ¶ ids a efficac f s d i es e ic ice i he f e ¶ s d .

S. aureus a d *C. albicans* e ¶¶ adhe¶i¶ hei ce ¶s face hich ¶¶ e¶ ¶i ia h ee di e¶i a ¶ha e [54] a d hich bi d ¶i ia e d acs a ¶s face (e.g. e d he ia ce¶ a d ¶s be d he ia a i ei¶) a d edica e s a -a id [54,55]. G i e he e¶i ia s is e ce echa i ¶, i i ¶s i g ha he ga i ¶ a¶ i fec a ie ¶ ih¶i ia i fac ¶, i cs di g ¶ - ea i ea d as a a ie ¶, a ie ¶ ih ce a e s¶ ca he e¶, a ie ¶ he dia ¶¶, a d a ie ¶ ih c ¶ ed hag c ich¶ defe ¶e echa i ¶ [4,56,57]. Fi a , s da a de ¶ a e ha he h¶ defe di¶e f agai¶ b h i fec i ¶ b ¶i ia echa i ¶, a d ha ada i e i s i b h ga i ¶ e$_O$s ied CD4+ T ce ds c i f b h IFN-γ a d IL-17A.

I ¶s a , he A¶3 -N acci e i ed s c ¶ i s¶ e de¶ f i *S. aureus* a d *C. albicans* i fec i b ids c i g s ¶ ea , -i fa a , Th1, Th17, a d Th1/17 h-c e, hich e ha ced ec si e a dac i ai f es hi¶ i i fec ed ¶¶s e, he eb eds c i g ¶¶s e i fec i s¶ bs de . Ths¶, acci a i ¶h ed a e ia ec agai¶ b h i fec i ¶ b a ge i g he ic be¶ f e ha ced de¶ s c i b i a e effec ce¶, i e ec e f es a i a i f ic bia is e ce fac ¶. The ef e, e ia acci e a ige¶ eed be e¶ ic ed ic bia is e ce fac ¶, a d ca be e a ded ic s de a a ge a ige hich e s ¶ i a e Th1 a d/Th17 i s e e ¶e agai¶ he ga i ¶.

Methods

Organisms and mouse strains

C. albicans SC5314 a ¶s ied b W. F i (Ge ge U i e¶i), a d *S. aureus* LAC, a USA300 MRSA c i ica i -a e, a ided b F a De e (NIAID/NIH). *C. albicans* 15563 i a c i ica b d ea i -a e f a a ie a Ha b -UCLA Medica Ce e hich i a¶ is e i s s ie de [50]. *Candida* a¶¶e ia a¶¶aged h ee i e¶ i ea e e de ¶e b h (Difc) a e e as e i i fec i . *S. aureus* a g e igh a 37°C i BHI b h, a d he a¶¶aged f 4 h s¶ a 37°C i f e¶h BHI b h.

Fe a e Ba b/c C57BL/6 ice e e b ai ed f Tac ic Fa ¶ (Be he da, MD). C ge ic IL-17A deficie ice a Ba b/c bac g s d e e b ai ed f Y. I a s a (U i e¶i f T) [58]. Vacci a ed ice e e i fec ed ia he ai ei ih he a ia e i cs a f *C. albicans* ba ¶ e *S. aureus* ga i ¶ i PBS, a s is¶ de c ibed [7,52]. I ¶ e e e i e¶, ice e e ade es e ic b ea e ih 230 g/ g c c h¶ ha ide 2 da ¶ i i fec i , a egi e hich e s ¶ i f s d es e ia f a i a e e ee [59,60]. A ceds e i s i g ice e e a s ed b he L¶ A ge e¶ Bi edica Re¶ ea ch I ¶ is e a i a s¶ e a d ca e c i ee, f i g he Na i a I ¶ is e f Hea h gside i e f a i a h s¶i g a d ca e.

Immunization protocols

A¶3 -N (a i acid 17 432 f A¶3) a ds ced i *Saccharomyces cerevisiae* a d s ified b Ni-NTA a i affi i s ifica i a s is¶ de c ibed [61]. Mice e e i s ied b ¶s bcs a e s¶ (SQ) i jec i f 300 µg f A¶3 -N i 0.1% A(OH)$_3$ (A h d ge, B e ag Bi ¶ec , F ede i¶¶s d, De-

a) i PBS. C ice ece ed adj a a e he a e ched e. S e ice e e b ed a 21 da . Mice e e i fec ed ee f i g he b .

Adoptive transfer and passive immunization

Se s a d e ic h c e e ha e ed f acci a ed c ice, a e ha e e is de c ibed [62]. L h de h c e e ha e ed f ce ica a da ia h de , ba ed i s die ih E a B s e d e h de a i g de ai g ha SQ acci ai a he ba e f he ec d ai ed i ai he e h de . F ad i e a fe , e ic a d h de h c e e ed. CD4+ T h c e e s ified b s e f he IMag e (BD Pha i ge), a e ha e de c ibed [6,7]. Ps ified h c e (5×10^6 e s e) e ead i ee di c ge ic, s acci a ed eci ie ice. T a fe ed ce s ai e e ≥95% s eb f c e ica a . Mice e e i fec ed ia he ai ei ih *C. albicans* SC5314 24 h afe h c e ad i e a fe .

Intracellular cytokine analysis and cytokine supernatant analysis

I ace s a c i e f h c e e a a ed ba ed a difica i f s e is de c ibed e h d [62]. I b ief, ce ica a da ia h de a d ee e e di ec ed f acci a ed c ice a d a ed h s gh 70 µ fi e . Ce e e i s a ed f 5 da ih A 3 -N (12.5 µg/) i c ee edia (RPMI 1640, 50 U/ e ici i , 50 µg/ e ci , 2 M L-gs a i e, 10% FBS, 5 µM 2-ME) i 96 e a e . PMA (50 g/), i ci (1 µM), a d e i (10 µg/) e e added ds i g he fi a 6 h s f cs s e. Ss e a a a ha e e ed i addi g e i f a a f c ie c e s ig C e ic Bead A a Fe i (BD Pha i ge , La J a, CA) ELISA (f IL-6, TGF-β, a d IL-13), e he a sfac s e i sci . Ce e e ai ed ice ih Pe CP-a i-CD4 a d A e a_{647}-a i-CCR6 (BD Pha i ge , Sa Dieg), hei i ec a ib die . The ce e fi ed a d e eabi i ed a e is de c ibed [62]. I ace s a c i e e ai ed ih a FITC-a i- s e IFN-γ a d PE-a i-IL-17, hei i ec (BD Pha i ge). F s -c f c e a e f ed a Bec -Dic i FACSca i s e ca ib a ed ih Ca iBRITE bead (Bec Dic i , Sa J e, CA) s ig FACSC f a e a e he a sfac s - e ec e da i . Da a f each a e e ac o_S ie ds i 10,000 $CD4^+$ h c e e a a ed. Th1 ce e e defi ed a $CD4^+IFN\gamma^+IL\text{-}17^-$, Th17 ce defi ed a $CD4^+IFN\text{-}\gamma^-IL\text{-}17^+$, a d Th1/17 ce defi ed $CD4^+IFN\gamma^+IL\text{-}17^+$.

Killing assay

The i i g a a f b h *C. albicans* a d *S. aureus* a dified ba ed s e -de c ibed a a [59,60]. I b ief, RAW s ie ac hage ce s ie es hi ce e e g i DMEM s 10% fe a b ie e s . F h s ie es hi e e ha e e ed b de a edi e a i f h e, he a a ied b d, f ed b ce if ga i e Fic H a o_S e a 500 g f 10 i s . The RAW ce es hi e e added i 24 e a , he edia i he e a a ia ed a d he RAW ce f h es hi e e cs s ed f 4 h s i 10% c di i ed edia (f acci a ed c e ic a d h de h c e e ed A 3 -N f 5 da) s 90% c ee edia (RPMI + 10% FBS). The c di i ed edia a he a i a ed, a d he ic ga i added he e i f h DMEM s 10% fe a b ie e s . Mic ga i e e added he e a a a i f 20:1 RAW ce *C. albicans*, 5:1 RAW ce *S. aureus*, 10:1 f h es hi *C. albicans* *S. aureus*. Media f he e c ai ig *S. aureus* c ai ed a ibi ic . The ce e e i cs ba ed a 37°C f 1 h, a hich i 4% b d hea i fs i (BHI) aga a di ec added he e . P a e e i cs ba ed e igh a 37°C a d c f i g s i (CFU) c s ed i each e . Ki i g a defi ed a he ce ed sc i CFU i e c ai i g c -cs s f hag c ce a d ic ga i c a ed e js c ai i g ic ga i .

Tissue burden, whole organ cytokines, myeloperoxidase (MPO), and histopathology

O da 4 -i fec i , id e (i a age ga) e e ha e ed a d h ge ie d i ai e ih ea e i hibi (e ai , es e i , a d PMFS). F de e i a i fi fec i s bs de , ga h ge a e e o_S a ia e cs s ed Sab s ad de e a ga f *C. albicans* ic aga f *S. aureus*. Wh e ga c i e e a a ed f id e h ge a e b ELISA (R&D S e) C e ic Bead A a Fe i f KC (BD Pha i ge , La J a, CA), e he a sfac s e i sci . MPO s e e e de e i ed b ELISA (H cs Bi ech g , Ude , Ne he a d) f h e ga h ge a e . F hi ah g , ga e e fi ed i ic-bs ffe ed f ai , e bedded i a affi , ec i ed, a d ai ed ih PAS f fs gi a d H&E a d G a ai f bac e ia.

Statistics

The - a a e ic L g Ra k a s ii ed de e i e diffe ce i s a i ci . The Wic Ra k a s ed c a ec i , MPO s e , a d ga bs de ac g s . $P<0.05$ a c ide ed ig ifica .

Supporting Information

Figure S1 Che he a -i ds ced es e ia ab a ed acci e i ds ced ec i agai a ec d *C. albicans* c i ica i a e. Si ee Ba b/c ice e g s e e acci a ed ih A 3 -N s A $(OH)_3$ A $(OH)_3$ a e, a d b ed h ee ee ae . T ee afe he b , haf he ice e e ea ed ih c c h ha ide. T da a e he ice e e i fec ed ih *C. albicans* 15563 (7 10^5). * <0.05 f acci a ed . c b L g Ra k .
F s da : d i:10.1371/j s a. a .1000703 001 (1.28 MB TIF)

Figure S2 FACS f ga i g Th1, Th17, a d Th1/17 ce i d ai i g h d . Sh he e a e e e a ie FACS , c e di g he da a i Fig. 6 f he a s ci , de ai g ac o_S i i ga e ba ed ie (FSC), de i (SSC), e e i f CD4 CCR6 he ce s face.
F s da : d i:10.1371/j s a. a .1000703 002 (1.85 MB TIF)

Figure S3 Vacci a i ed Th1, Th17, a d Th1/17 ce i d ai i g h d . FACS de ai g a a f c ie e e i a g h c e s ig he ga e h i Fig. S2.
F s da : d i:10.1371/j s a. a .1000703 003 (1.52 MB TIF)

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Author Contributions

C ce ed a d de ig ed he e i e : LL ASI JMF BS. Pe f ed he e i e : LL ASI XX VA BB YF BS. A a ed he da a: LL SWF BS. C ib ed eage / a e ia / a a : XX JMF VA YF JEEJ BS. W e he a e : LL BS.

References

1. Wi i gh ff H, Bi ch ff T, Ta e SM, Seife H, We e RP, e a . (2004) N c ia b d ea i fec i i US h i a : a a f 24,179 ca e f a ec i e a i ide s ei a ce s d . C i I fec Di 39: 309 317.
2. Cha be HF (2005) C s i -a cia ed MRSA e i a ce a d i e ce c e ge. N E g J Med 352: 1485 1487.
3. Wi LS, Re e CM, S a M, S ec a J, A e K, e a . (2002) The di ec c a d i cide ce f e ic f ga i fec i . Va e Hea h 5: 26 34.
4. S e be g B, Fi e SG, Ed a d JE J (2006) C e T ea e S a egie f Di e i a ed Ca didia i . C i I fec Di 42: 244 251.
5. Li L, Ib ahi AS, A a e ia V, Ed a d JEJ, F Y, e a . (2008) Vacci e i s ge ici a d efficac a c ide ab b di e s ed f a s i s h d ide adj a . C i Vacci e I s 15: 582 584.
6. S e be g B, Ib ahi AS, Li L, A a e ia V, F Y, e a . (2008) A a ib d i e h e h d edic a i-ca dida acci e efficac e e h s gh he echa f ec i i d c i f ce - edia ed i s i . J I fec Di 197: 957 971.
7. S e be g B, Ib ahi AS, Yea a M, Li L, F Y, e a . (2008) The a i-f ga A 3 -N acci e ec ice agai he bac e i S a h c cc a e . I fec I s 76: 4574 4580.
8. Dje JY, B a cha d DK (1987) Reg a i f h a h s c ea e hi (PMN) ac i i agai Ca dida a bica b a ge g a s a h c e ia e ea e f a PMN-ac i a i g fac . J I s 139: 2761 2767.
9. P e a L, D g DH, T ebicha I, F a a E, S e a a V, e a . (1994) A ibac e ia ac i i f h a s c ea es c e agai S a h c cc a e . F ia Mic bi (P aha) 39: 428 434.
10. Leijh PC, a de Ba e aa MT, a F h R (1977) Ki e ic f hag c i a d i ace s a i i g f Ca dida a bica b h a g a s c e a d c e. I fec I s 17: 313 318.
11. Dje JY, B a cha d DK, Ha ia D, F ied a H (1986) G h i hibi i f Ca dida a bica b h a h s c ea es hi : ac i a i b i e fe -ga a a d s ec fac . J I s 137: 2980 2984.
12. Ta chdjia CL, T i EF, H KC, See ig MS, C e a MB, e a . (1971) I s f e ce ce s die f ca dida i h a e ic e d he ia hag - c e: i ica i f i s ge e a d a h ge e f e ic ca didia i . A J C i Pa h 56: 50 58.
13. Mei e H, He e B, Schafe H, Hafe a O (1977) R e f Ca dida a bica i g a s a s s e eac i . II. I i deg ada i f C. a bica i he a ic ac hage f ice. J I fec Di 135: 235 242.
14. DeCha e e LR, Mig e RA, Shi e PS, M HB, S ejda P, e a . (1978) C a f i ace s a bac e icida ac i i e f h a es hi a d e i hi . B d 52: 609 617.
15. Ma de GL (1975) Ca a a e, s e ide di s a e, a d i s e ce f S a h c cc a e . I i a d i s die i h e ha a h c cca es c e i e ac i . J C i I e 55: 561 566.
16. Ma de GL, R bi W, H EW (1970) The effec f a NADH ida e i hibi (h d c e) h s c ea es c e bac e icida ac i i . J C i I e 49: 1381 1388.
17. Dia d RD, Ha de chi d CC (1981) M c e- edia ed e s -i de e - de da age h ha a d es d h ha f f Ca dida a bica i i . J C i I e 67: 173 182.
18. Dia d RD, K e ic i R (1978) Mecha f a ach e f es hi Ca dida a bica es d h hae i he ab e ce f e s , a d f s b e s e da age es d h hae b ic bicida ce e f es hi i i . J C i I e 61: 360 369.
19. Dia d RD, K e ic i R, Ja W (1978) Da age es d h ha f f Ca dida a bica b es hi i he ab e ce f e s i i . J C i I e 61: 349 359.

53. S e be g BJ, Ib ahi AS, A e ia V, Fi e SG, M e CL, e a. (2005) The a i-Ca dida a bica s acci e c ed f he ec bi a N e is f A l eds ce fs ga bs de a di s s a i b hi s c ee a di s c ed ice. I fec I s 73: 6191 6193.
54. She a d DC, Yea a MR, We ch WH, Pha QT, Fs Y, e a. (2004) Fs ci a a d s cs a di e i he A ei fa i f Ca dida a bica . J Bi Che 279: 30480 30489.
55. Pa i JM, A e BL, McGa i MJ, H M (1994) MSCRAMM- edia ed adhe e ce f ic ga i h s ms . A s Rs Mic bi 48: 585 617.
56. Las a d KB, Chs ch DL, Ms ce i M, Ss he a d LR, Da ie HD (2003) P s ai -ba ed s d f he e ide i g f a d he i fac f i a ai e S a h c ccs as es i fec i . J I fec Di 187: 1452 1459.
57. Je e AG, Wach a CH, P s e KB, Bi e e F, Scheibe J, e a. (1999) Ri fac f h ia-ac os ied S a h c ccs as es bac e e ia. A ch I e Med 159: 1437 1444.
58. Na ae S, K i a a Y, Na bs A, Ss d K, I a e M, e a. (2002) A ige - ecific T ce e i i ai i ai ed i IL-17-deficie ice, cas ig s ci f a e gic ce s a a d hs a d . I s i 17: 375 387.
59. S e be g BJ, C i M, A a ia V, G e M, Ed a d JE J, e a. (2006) O i i ai f a e id ce a fs i a eg f i fec ed es e ic h . J Les c Bi .
60. S e be g BJ, C i M, F e ch SW, Ed a d JE J, Fs Y, e a. (2005) A hag c ic ce i e a ed i s s a i fec ed es e ic ice. J Les c Bi 78: 338 344.
61. S e be g BJ, Ib ahi AS, A a ia V, Fs Y, M e C, e a. (2006) Efficac f he a i-Ca dida A 3 -N A l -N s acci agai dis e i a ed a d sc a ca didias . J I fec Di 194: 256 260.
62. S e be g BJ, J h D, Pha QT, Ed a d JEJ, F e ch SW, e a. (2003) Pa e ch a ga , a d e ic, i s i c ea h ih s s a ds i g dis e i a ed ca didias . I fec I s 71: 5756 5764.