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Critical role of IL22/IL22RA1 signaling in pneumococcal pneumonia

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Abstract

IL-22-IL-22R signaling plays a crucial role in regulating host defenses against extracellular pathogens particularly in the intestine, through the induction of antimicrobial peptides and chemotactic genes. However, the role of IL-22- IL-22R is understudied in Spn lung infection, a prevalent pathogen of pneumonia. This paper presents the findings of IL-22 signaling during a murine model of pneumococcal pneumonia and improvement of bacterial burden upon IL-22 administration. IL-22 was rapidly induced in the lung during pneumococcal infection in wild type mice and *Il22*$^{-/-}$ mice had higher pneumococcal burdens compared to controls. Additionally, mice with hepatic-specific deletion of *Il22ra1* also had higher bacterial burdens in lungs compared to littermate controls after intrapulmonary pneumococcal infection, suggesting that IL-22 signaling in the liver is important to control pneumococcal pneumonia. Thus, we hypothesized that enhancement of IL-22 signaling would control pneumococcal burden in lung tissues in an experimental pneumonia model. Administration of recombinant IL-22 systemically to infected wild type mice decreased bacterial burden in lung and liver at 24 hours post infection. Our in vitro studies also showed that mice treated with IL-22 had increased C3 expression in the liver compared to the isotype control group. Furthermore, serum from mice treated with IL-22 had improved opsonic capacity by increasing C3 binding on Spn. Taken together, endogenous IL-22 and hepatic IL-22R signaling play critical roles in controlling pneumococcal lung burden and systemic IL-22 decreases bacterial burden in the lungs and peripheral organs by potentiating C3 opsonization on bacterial surfaces, through the increase of hepatic C3 expression.

Introduction

IL-22 is a secreted cytokine produced mainly by cells of the lymphoid lineage and innate lymphoid cells (1). Ligation of IL-22 to IL-22RA1 and IL-10R2 results in activation of JAK1/TYK2 kinases and downstream phosphorylation of STAT proteins, in particular STAT3 (2, 3). IL-22RA1 is characteristically expressed in epithelial cells, and its activation by IL-22 is involved in host defense against extracellular organisms including *Klebsiella pneumoniae* (4) *Citrobacter rodentium* (5, 6) and *Candida albicans* (7). Also, IL-22 signaling is related to maintaining barrier integrity and epithelial repair during influenza infection (8), mediating recruitment of leukocytes, and inducing antimicrobial peptides. IL-22RA1 is also

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abundantly expressed in hepatocytes and has been shown that IL-22 liver signaling is critical for liver repair in acute injury models such as acute alcoholic hepatitis (9) as well as fibrotic injury such as in exposure to carbon tetrachloride (10). Furthermore, IL-22 promotes resistance against pathobionts by regulating the complement system (11) in a mouse model of *Clostridium difficile* colitis.

However, the role of IL-22 is understudied during pneumococcal pneumonia. *Streptococcus pneumoniae* (Spn) accounts for 4 million illness episodes, 445,000 hospitalizations and 22,000 deaths annually in the US (12). Children younger than 2 years old (12-31 cases/100,000) and individuals older than 65 years (~37 cases/100,000) are the most vulnerable for invasive disease. Although much work has been done on investigating the local pulmonary response to control Spn infection, it has been shown that also the liver is key to pneumococcal host immunity by controlling bacterial burden and dissemination (13). When both, NF-κB RelA and STAT3 are selectively deleted in hepatocytes, mice showed higher pneumococcal burdens particularly in extrapulmonary tissues (13). Several cytokines have been shown to be critical for controlling this hepatic response in the infected lung including TNF-α, IL-1β and IL-6. The fact that the liver has an important role in controlling pneumococcal pneumonia is also supported by clinical evidence. In particular, patients with liver cirrhosis (alcoholic and non-alcoholic) have higher rates of pneumococcal pneumonia as well as a higher incidence of pneumococcal bacteremia and extrapulmonary dissemination (14, 15).

Having the evidence that IL-22 is important for the host response against certain extracellular pathogens as well as having evidence of IL-22RA1 expression in the liver, we first confirmed that IL-22 was induced in the lung after a pneumococcal lung infection model in wild type mice. We confirmed that IL-22KO mice were more susceptible to infection compared to control mice, which suggested that endogenous IL-22 played a role in controlling pneumococcal burden. Thus, we further hypothesized that enhancement of IL-22 signaling would control pneumococcal burden in lung tissues in an experimental pneumonia model. This paper describes the increased pneumococcal susceptibility in mice that have a liver specific deletion of IL-22RA1 as well as the improved bacterial burden not only in lung but also in extrapulmonary tissues after recombinant IL-22 administration. Complement component 3 (C3) was increased in liver tissues from mice treated with IL-22 compared to the isotype control group and serum from mice treated with IL-22 had improved opsonic capacity by increasing C3 binding on Spn. These findings suggest the importance of local IL-22 production upon pneumococcal infection, IL-22RA1 hepatic signaling in controlling bacterial burden in part by increasing hepatic C3 expression and increased C3 deposition on pneumococci, which translates into decreased bacterial burden in the host.

Material and Methods

Mice

***Il22ra1*$^{fl/fl}$ mice** on a C57BL/6 background were designed in our laboratory and made at Ozgene. LoxP sites were engineered to flank exon 3. To determine functional recombination, *Il22ra1*$^{fl/fl}$ mice were injected in the tail vein with an E1-deleted adenovirus expressing Cre recombinase and liver DNA was extracted and subjected to PCR using

primers spanning exon 3. To determine functional deletion of IL-22RA1 specifically in the liver of *Il22ra1*$^{fl/fl}$ mice, we crossed this line to *B6.Cg-Tg(Alb-cre)21Mgn/J* or ***Albumin-Cre*** **mice** (obtained from The Jackson Laboratory), which confirmed cre-mediated DNA recombination by allele specific PCR. ***Ccsp-Cre*** **mice** were obtained as a kind gift from Dr. Thomas Mariani and described before(16). ***Il22***$^{-/-}$ **mice** were obtained from The Taconic Farms.

Animal Studies

8-10 week old mice were used for in vivo studies. All animal studies were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Experimental Spn mouse infection

One fresh colony of Spn serotype 3 (ATCC 6303) was grown in 100 ml of Todd-Hewitt broth for 10 hours at 37°C, 5%CO_2. 1 ml aliquots were obtained and centrifuged at 17000 g for 1 minute and washed twice with PBS. Bacterial pellet was resuspended in PBS and concentration was determined by measuring the absorbance at 600 nm. An absorbance of 0.5 was about 2×10^8 CFU/ml. An inoculum of 10^6 CFU/mouse was given by intranasal aspiration (in 50 ul of PBS). The working stock was serially diluted and plated on trypticase soy agar with 5% sheep blood plates (TSAII) to determine the exact concentration of the inoculum.

Recombinant proteins

IL22:Fc 4 ug/mouse (Generon, Shanghai, China) or isotype control 4 ug/mouse (human IgG) were given by intraperitoneal injection 3-4 hours before Spn infection. Anti IL-23p19: 500ug/mouse by intraperitoneal injection right before infection with Spn. Anakinra: 100 mg/kg by intraperitoneal injection right before infection with Spn. Cobra venom factor (CVF) (EMD Millipore): 25 ug/mouse of CVF was administered intraperitoneally for 24 hours before infection with Spn.

Bacterial burden, mRNA, and protein analysis

To determine bacterial burden, lungs, livers and spleen were harvested in PBS, homogenized with glass homogenizer, and plated on TSA II plates in four serial dilutions. Colonies were counted after overnight incubation at 37°C, 5%CO2. To determine gene expression, lungs and livers were placed in TRIzol reagent (Invitrogen), homogenized and processed according to manufacturer's protocol. One microgram of RNA was used to synthesize cDNA. Real-time PCR primers with SSOFast Probes Supermix probes were used. Threshold cycle (C_T) values were normalized to *Hprt*. For protein analysis, tissues were placed in RIPA cell lysis buffer with protease inhibitor mixture tablets (Roche Applied Science) homogenized, and centrifuged at 10,000 3 g. IL-22 levels in supernatants were measured by ELISA (eBioscience) and normalized to total protein levels (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific).

C3 binding assay

Serum was collected from mice 24h after i.p administration of IL-22:Fc or isotype control . 10^6 CFU of Spn were incubated for 20 min at 37°C (no CO2), with no rotation with 10 or 50 ul of serum (from IL-22:Fc treated mice or isotype control), followed by washing with PBS Tween 0.1% . Mix of bacteria and serum was resuspended FITC-conjugated anti-mouse C3 (1:300; MBL International) for 20 min at 4°C. The percentage of positively labeled bacteria was determined using flow cytometry. Background fluorescence was based on that exhibited by non- opsonized bacteria.

Lung cell sorting and Flow Cytometry

Lungs were digested with collagenase D (3 mg/ml) in PBS for 1 h at 37°C, crushed through 100-mm cell strainers, and treated with ammonium chloride to lyse RBCs . Single cell suspensions were stained with CD16/32 (eBioscience). Two pooled lungs of *Spn* infected mice were stained with fluorochrome-conjugated antibodies of the following surface antigens: CD4, CD8α, and TCR-γδ . Three pooled lungs of infected mice with *Spn* were stained with fluorochrome-conjugated antibodies of the following surface antigens: CD11c, CD11b, F4/80, CD19, Ter119, NK1.1, TCR-β, TCR-γδ, CD8b, CD4 and CD90.2. Live / dead fixable blue cell stain kit was used to exclude dead cells. For neutrophil staining, the following fluorochrome-conjugated antibodies were used: CD45, Ly6G, Cd11b and F4/80. Gating strategies are supplied in Supplement Figure S1 (A-C).

Statistical Analyses

Unpaired, one-tailed, Student's t tests, $p< 0.05$, were used to assess whether the means of two normally distributed groups differed significantly. ANOVA or Kruskal-Wallis was used to compare multiple means. Significance is indicated as $p <0.05$.

Results

IL-22 is required for host control of Spn pulmonary infection

To assess whether *Il22* is expressed upon pneumococcal pneumonia, wild type C57Bl/6 mice (B6) were infected with 10^6 CFU of Spn serotype 3 and groups were sacrificed at different time points: 5h, 12h, 24h and 48h. Bacterial burden had a direct correlation with time of infection, thus, the highest bacterial burden in the lungs was at 48 hours post infection, after which we observed more than 95% mortality (data not shown). Lung *Il22* expression increased as early as 5 hours post infection (Figure 1A) and even though, liver bacterial burdens increased significantly at 48 hours, hepatic *Il22* gene expression was undetectable across all time points (Figure 1B), which suggests that the liver does not produce IL-22 upon pneumococcal lung infection. Furthermore, IL-22 protein was detected in lung homogenates at 12 hours post infection and it was also present in serum (Figure 1C) at the same time point. To evaluate if endogenous IL-22 is required for control of Spn infection we infected *IL22*$^{-/-}$ mice or control B6 mice and sacrificed them at 24 hours post infection. *IL22*$^{-/-}$ mice had increased bacterial burdens at 24 hours post infection compared to B6 mice in both the lung and liver (Figure 1D). Thus, endogenous IL-22 is required for control of pneumococcal burden after intrapulmonary infection.

IL-22 is regulated by Spn induced IL-23 and IL-1β

IL-23 and IL-1β, which are produced by macrophages and dendritic cells, are induced during pneumococcal pneumonia (17, 18). These two cytokines are major inducers of IL-22 (19). To asses if IL-23 and IL-1R control IL-22 expression in the pneumococcal pneumonia model, mice received anti-IL23 subunit p19 antibody (anti IL-23p19), IL-1R antagonist (IL-1Ra) or isotype control by intraperitoneal administration, followed by Spn infection. Mice were sacrificed at 5 hours post infection, since at this time point lung bacterial burdens were similar among experimental groups. At 5 hours post infection the anti-IL-23p19 group had substantially decreased *Il22, Lcn2 and Reg3g* compared to the isotype control group (Figure 2). Mice that received IL-1Ra prior to pneumococcal infection also displayed decreased *Il22* mRNA expression as well as, *Il17 and Lcn2* compared to the control group.

Cellular source of *Il22* during acute pneumococcal pneumonia

To evaluate the cellular sources of IL-22 during acute pneumococcal pneumonia, B6 mice were infected with Spn (10^6 CFU/mouse) and sacrificed 24 hours post infection. Single cell suspensions from lung tissue were stained and flow cytometry sorted for the following cell populations: $CD4^+$, TCR-$\gamma\delta^+$ and Lin^- $CD90.2^+$ The sorted groups of cells were evaluated for expression of *Rorc, Il22, Il17, Gata3, Il13, Il5* and *Ifng* (Figure 3). In the lung, TCR-$\gamma\delta^+$ cells showed the highest expression of *Il22*, followed by Lin^- $CD90.2^+$ cells, which likely represent innate lymphoid cells. Although our sorting strategy for ILCs did not differentiate between ILC2 and ILC3, genes expressed in ILC2 like *Gata3, Il13* and *Il5* were highly expressed in our sorted Lin^- $CD90.2^+$ lung cells, suggesting that this group may represent mostly ILC2 cells with a smaller population of ILC3 cells, which expressed *Rorc* and *Il22*. Thus, in this acute pneumococcal pneumonia model TCR-$\gamma\delta^+$ cells were the predominant IL-22 producing cells. The frequency (percentage) and absolute number of cells were similar in sorted TCR-$\gamma\delta^+$ and $Lin^-CD90.2^+$ (Supplement Table S1).

IL-22RA1 signaling in the liver is required for control of pneumococcal pneumonia burden

We evaluated the requirement of IL-22RA1 in lung epithelium in our pneumococcal pneumonia model. For this purpose, conditional lung epithelium specific *Il22ra1* deficient mice: *Ccsp Cre*$^{+/-}$ *Il22ra1*$^{fl/fl}$ (*Il22ra1*$^{\Delta Ccsp}$) and *Ccsp Cre*$^{-/-}$ *Il22ra1*$^{fl/fl}$ (*Il22ra1*$^{fl/fl}$) littermate control were used. Upon experimental lung infection, there was no difference in lung bacterial burden between *Il22ra1*$^{fl/fl}$ *and Il22ra1*$^{\Delta Ccsp}$ groups (Figure 4A), which suggested that IL-22 signaling in the CCSP+ cells is dispensable in controlling lung pneumococcal burden. As mentioned before, IL-22RA1 is highly expressed in the liver and can activate STAT3 (3, 20, 21). Thus, to address the contribution of hepatic IL-22RA1 signaling during pneumococcal lung infection, we generated a conditional liver specific *Il22ra1* knockout: *Alb-Cre*$^{+/-}$ *Il22ra1*$^{fl/fl}$ (*Il22ra1*$^{\Delta HEP}$) and used *Alb-Cre*$^{-/-}$ *Il22ra1*$^{fl/fl}$ (*Il22ra1*$^{fl/fl}$) as littermate controls (22). Upon experimental lung infection *Il22ra1*$^{\Delta HEP}$ mice had increased bacterial burdens in the lung and liver at 24 hours post pneumococcal infection compared to *Il22ra1*$^{fl/fl}$ mice (Figure 4B).

IL-22 pre-treatment controls pneumococcal burden in lung tissue

Next, we determined if administration of exogenous IL-22 could lead to improved host bacterial burden. B6 mice were pretreated with IL-22:Fc or isotype control , followed by Spn infection (10^6 CFU/mouse) and sacrificed at 24 hours post infection. B6 mice that received pre-treatment with IL-22:Fc showed decreased lung, liver and spleen bacterial burdens at 24 hours post pneumococcal lung infection, compared to the isotype control group (Figure 5A). Gene expression analysis in lung tissues showed no difference in genes reported to be regulated by IL-22 including Lcn2, *Reg3g*, *Saa2/1* or *Saa3* (Figure 5B). On the other hand, in liver tissue, *Saa2/1* was increased in the IL-22Fc group compared to isotype control group, while other genes such as *Saa3* and *Lcn2* were not different between groups (Figure 5C).

IL-22 increases hepatic C3 expression and serum opsonization capacity

Since pretreatment with IL-22:Fc decreased bacterial burden compared to isotype control and since IL-22RA1 in the liver is important for pneumococcal burden control, we first tested if IL-22:Fc would increase gene expression of Complement component 3 (C3) in liver tissue. For that B6 mice received either Isotype or IL-22Fc for 24 hours. Afterwards, liver tissues were retrieved and assessed for C3 gene and protein expression. IL-22:Fc treated mice had higher C3 expression in liver tissues compared to isotype group (Figure 6A). Furthermore, to asses if C3 would be contributing to increase bacterial opsonization, we performed an in vitro C3 binding assay, in which Spn was opsonized with serum from IL-22:Fc or Isotype treated mice. There was increase in C3 binding of Spn opsonized with IL-22:Fc serum compared to the Isotype control (Figure 6B). To determine the role of endogenous C3 upon infection, C3 was depleted in vivo by the administration of cobra venom factor (CVF) for 24 hours or PBS as control. These groups of mice were further randomized to IL-22:Fc or isotype pre-treatment for 3 hours, followed by Spn (10^6 CFU/mouse) and sacrificed at 24 hours post infection. Mice that received CVF and IL-22Fc had some decrease in lung bacterial burden compared to the group that received CVF and isotype (Figure 6C), but this difference did not reach statistical significance, which suggested that IL-22 had a partial rescue in bacterial burden.

Discussion

In this study we investigated if IL-22 plays a role during pneumococcal pneumonia, hepatic IL-22RA1 signaling for control of bacterial burden and whether exogenous IL-22 administration offers protection to the infected host. Our findings show that IL-22 is required for control of pneumococcal burden, which is supported by *Il22* gene expression in the lungs as early as 5 hours post infection. *Il22* is expressed in the lungs along with other cytokines like *Il6, Il1β* and *Tnfα* during pneumococcal pneumonia. We showed that genetic deletion of IL-22 makes the host more susceptible, which supports the role of IL-22 in controlling pneumococcal burden. We also found that IL-22 produced during pneumococcal pneumonia is dependent on IL-23 and IL-1β signaling. This finding is supported by the well established role of IL-23 and IL-1β in controlling IL-22 expression using different models and routes of infection (4, 5).

In our experimental model, TCR- $\gamma\delta^+$ cells were the predominant source of *Il22* during the early stages of pneumococcal pneumonia. This was supported by higher gene expression of *Il22, Rorc* and *Il17* in flow cytometry sorted TCR- $\gamma\delta^+$ cells from the lungs of infected mice. The second population that expressed *Il22* were ILCs (likely ILC3), although due to the small number of cells, we did not differentiate further between ILC2 and ILC3. Based on the higher levels of *Gata3, Il13* and *Il5* in this population, we assume it mostly represents ILC2 cells. ILC3 cells have been described to produce IL-22 during pneumococcal pneumonia (23), although in our study, the sorted population were not cultured ex-vivo for 3 days as reported by this other group. As the lungs have very low numbers of TCR- $\gamma\delta^+$ cells and ILCs (24) in the lungs, we had to pool samples to overcome this issue and have adequate material for gene expression analysis.

In our pneumococcal infection model, the lack of IL-22RA1 signaling in the liver resulted in a higher bacterial burden after pulmonary inoculation. This suggests that engagement of IL-22 and hepatic IL-22RA1 is important for controlling pneumococcal lung burden and dissemination. Furthermore, pre-treatment with systemic exogenous IL-22 (IL-22:Fc) resulted in decreased bacterial burden in lung and peripheral tissues. The importance of the liver in controlling lung bacterial burden goes in line with previous studies that have found the importance of the liver response to control bacterial pneumonia (25, 26), as hepatic specific mutations of both STAT3 and NF-κB RelA abrogate the hepatic acute phase response and renders the host more susceptible to bacterial dissemination (13, 27). IL-22, also a potent acute phase reactant inducer (28, 29) is induced during pneumococcal pneumonia and in physiologic amounts likely acts in concert along with other cytokines such as IL-6, IL-1β, TNF-α. Nevertheless, contrary to other secreted cytokine's receptors, IL-22RA1 is present at specific mucosal barrier sites, in non-immune cells, which makes its activity more targeted and potentially with less collateral damage when used as a therapeutic agent to help controlling pneumococcal pneumonia and dissemination. We also assessed if pretreatment with IL-IL-22:Fc in mice infected with a lower dose of Spn (LD50: 10^3CFU/mouse) would confer some advantage in survival at 10 days post infection (60% for IL-22:Fc treated versus 20% for isotype control) but this did not reach statistical significance. (Supplement Figure S2).

One of the mechanisms by which pretreatment with IL-22: Fc decreases bacterial burden at 24 hours in our study is by improving Complement factor 3 (C3) deposition on pneumococci. Recent evidence supports the role of IL-22 in regulating the complement system (11) and eliminating commensal bacteria during *C. difficile* infection, thus our finding goes in line with this report. Furthermore, our in vivo C3 depletion studies, using CVF showed that pretreatment with IL-22:Fc was associated with a slight reduction in bacterial burden in the lungs, although this did not reach statistical significance. This may suggest additional mechanisms in our model by which IL-22 is contributing to bacterial containment. To this end, one aspect to explore is the role of SAA 1 and 2 in our infection model, since they are markedly increased by IL-22 in our model (Figure 5C). The increase in C3 deposition may contribute to improved neutrophil phagocytosis. Thus we first tested if neutrophil recruitment was affected in the IL-22:Fc group compared to isotype control mice. Neutrophil recruitment into the BAL fluid was similar in both groups (Supplement Figure S3A-B). To determine the uptake of Spn we sorted $Ly6G^+$,$Cd11b^+$, $F4/80^-$ cells from total

lungs (which represent lung neutrophils) and extracted total RNA from these cells. Sorted neutrophils from IL-22:Fc treated mice had increased detectable *CpsA* gene expression (a gene specifc for Spn, Supplement Figure S3C)(30), which suggests that neutrophils from the IL-22:Fc treated mice had improved bacterial phagocytosis consistent with the observed increase in C3 bacterial deposition. Furthermore, the Fc part of IL-22:Fc itself seems not to activate C3, as we retrieved sera from naïve mice and spiked them either with IL-22:Fc or Isotype control at 2ug/ml. Afterwards these spiked sera was used to opsonize Spn at 37oC for 20 minutes. C3 binding capacity was similar in both groups, suggesting that Fc portion of IL-22:Fc may not activate C3 in vitro (Supplement Figure S3D).

In conclusion, endogenous IL-22 plays a role in protecting the host during pneumococcal pneumonia and enhancement of IL-22-IL-22RA1 signaling is beneficial for the host to control pneumococcal pneumonia. IL-22:Fc which is currently in clinical trials for gut graft versus host disease (NCT02406651) and alcoholic hepatitis (NCT02655510) may be a useful adjunct therapy for severe pneumococcal infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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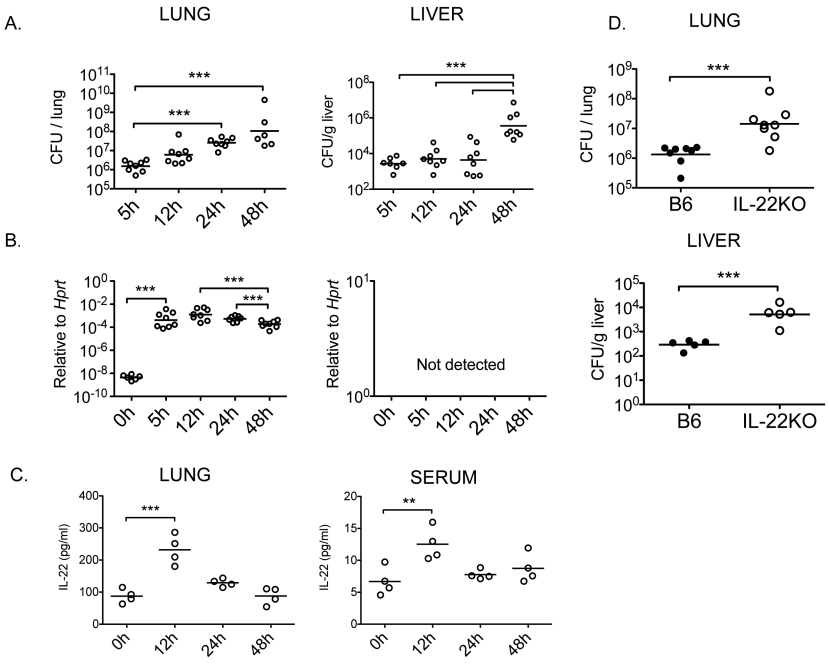

Figure 1. IL-22 is required for host control of Spn pulmonary infection

A) Spn burden in lungs and livers at different time points. Mice were infected with Spn 10^6 CFU/mouse by oropharyngeal aspiration and groups were sacrificed at different time points. B) *Il22* gene expression in lungs and livers. C) IL-22 protein expression detected by ELISA at different times points of infection in lung tissue homogenate and serum. * $p < 0.05$, **$p<0.01$, *** $p<0.001$ (One-way ANOVA). D) Bacterial burden in lung and liver between B6 and IL-22 knockout (IL-22KO) mice. *** $p<0.001$ (unpaired t-test). Each circle represents one mouse. Experiments were repeated two times.

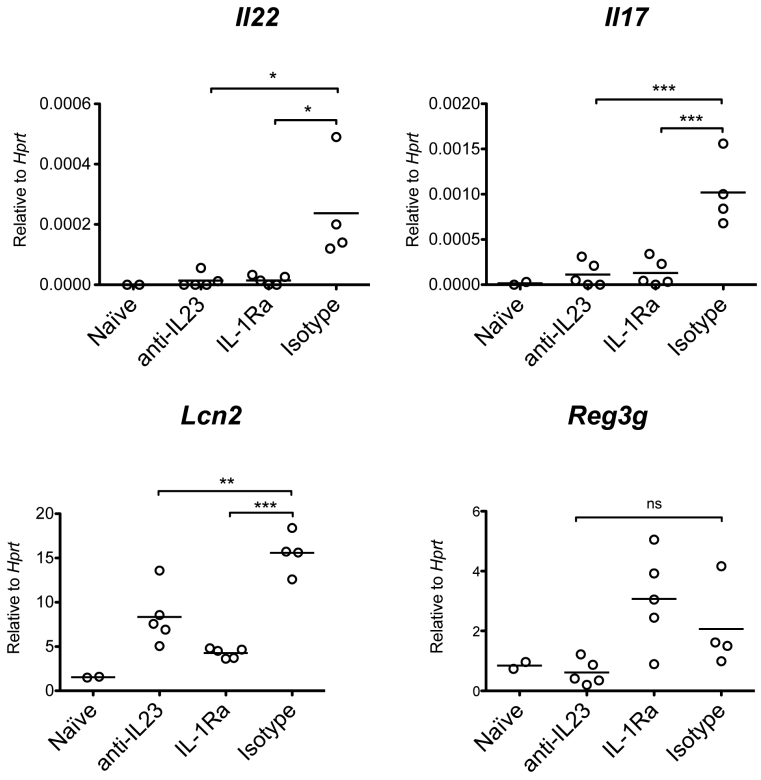

Figure 2. IL-22 production is dependent on IL-23 and IL-1β upon Spn pneumonia

Wild type mice received either anti IL-23 p19, IL-1Ra or isotype control. Mice were sacrificed at 5 hours post infection. Gene expression of *Il22*, *Il17*, *Lcn2* and *Reg3g* were assessed by qPCR. Each circle represents one mouse. * p <0.05, **p<0.01, *** p<0.001 (One-way ANOVA).

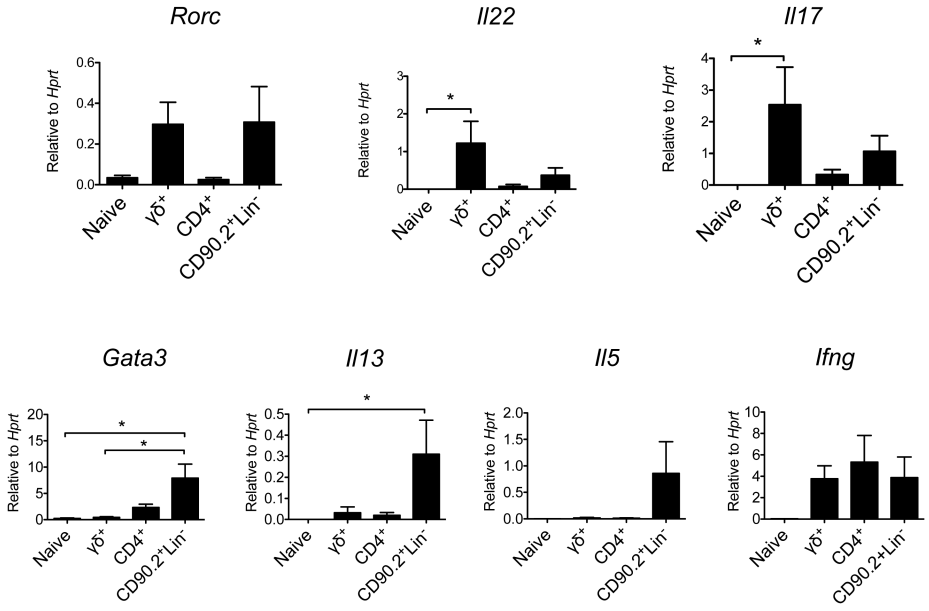

Figure 3. Cellular source of IL-22 during pneumococcal pneumonia

Mice were infected with Spn for 24 hours. Lungs were collected and collagenase treated for single cell suspension. Two pooled lungs of *Spn* infected mice were stained with CD4, CD8, and TCR-γδ antibodies; CD4+ cells and TCR-$\gamma\delta^+$ cells were sorted. Three pooled lungs of infected mice with *Spn* were stained with Lin (CD11c, CD11b, F4/80, CD19, Ter119, NK1.1, TCRβ, CD8, CD4) and CD90.2 antibodies; Lin^- $CD90.2^+$ cells were sorted. Cell populations underwent gene expression by qPCR. Data represent three separate experiments. * $p < 0.05$ (One-way ANOVA).

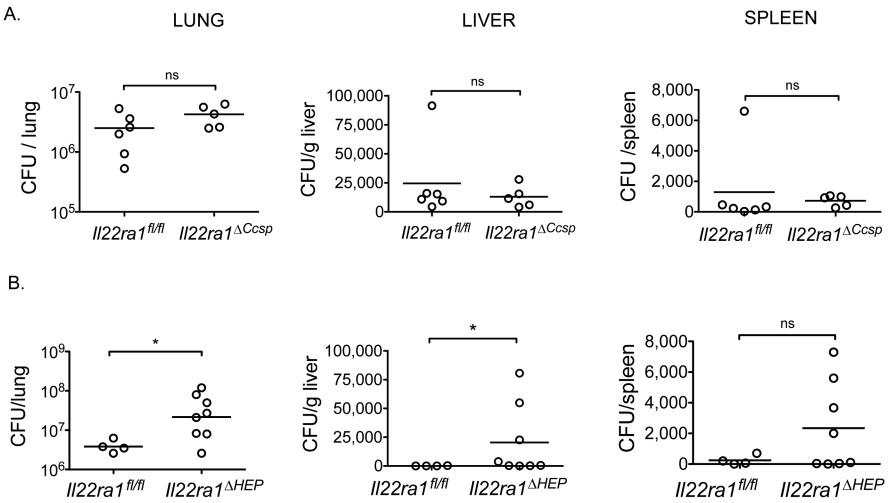

Figure 4. IL-22RA1 signaling in the liver is required for control of pneumococcal pneumonia burden

A) *CcspCre*$^{-/-}$ × *Il22ra1*$^{fl/fl}$ *(Il22ra1*$^{fl/fl}$*)* and *CcspCre*$^{-/+}$ × *Il22ra1*$^{fl/fl}$ *(Il22ra1*$^{\Delta Ccsp}$*)* mice were infected with Spn 10^6 CFU/mouse and sacrificed at 24 hours post infection. B) AlbCre$^{-/-}$ × Il22ra1$^{fl/fl}$ (Il22ra1$^{fl/fl}$) and AlbCre$^{-/+}$ × Il22ra1$^{fl/fl}$ (Il22ra1$^{\Delta HEP}$) were infected with Spn 10^6 CFU/mouse and sacrificed at 24 hours post infection. *p<0.05 , ns: p>0.05 (unpaired t-test). Each circle represents one mouse. Experiments have been repeated twice.

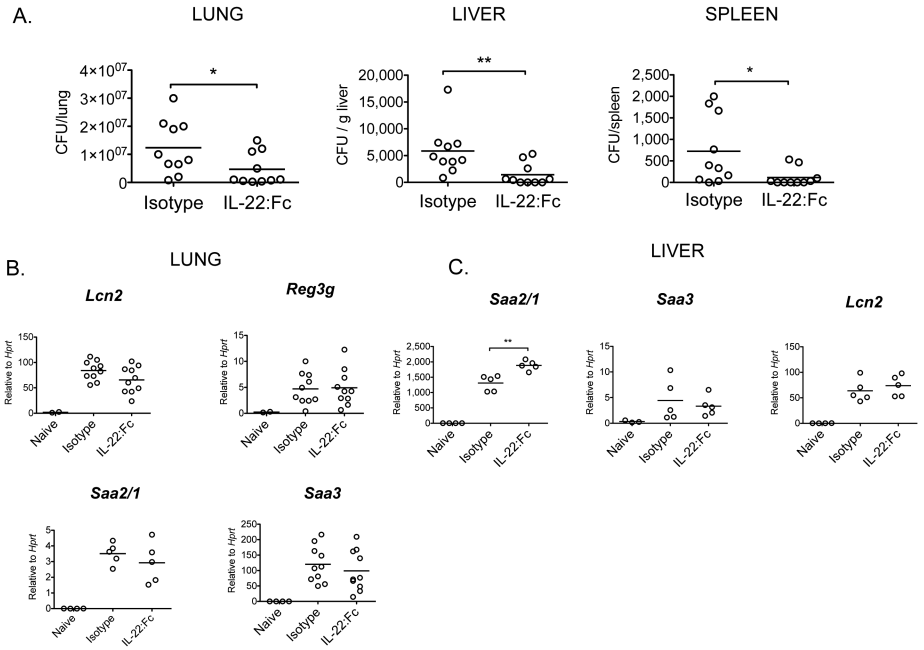

Figure 5. IL-22 pre-treatment controls pneumococcal burden in lung tissue

A) Wild type mice were given 4ug of IL-22:Fc or Isotype by intraperitoneal route 4 hours before Spn infection (10^6 CFU/mouse) and sacrificed at 24 hours post infection. Each circle represents one mouse. *p< 0.05, **p< 0.01 (unpaired t-test). Gene expression in B) lungs and C) livers of naïve , Isotype or IL-22:Fc pre-treated mice followed by Spn infection. **p<0.01(One-way ANOVA).

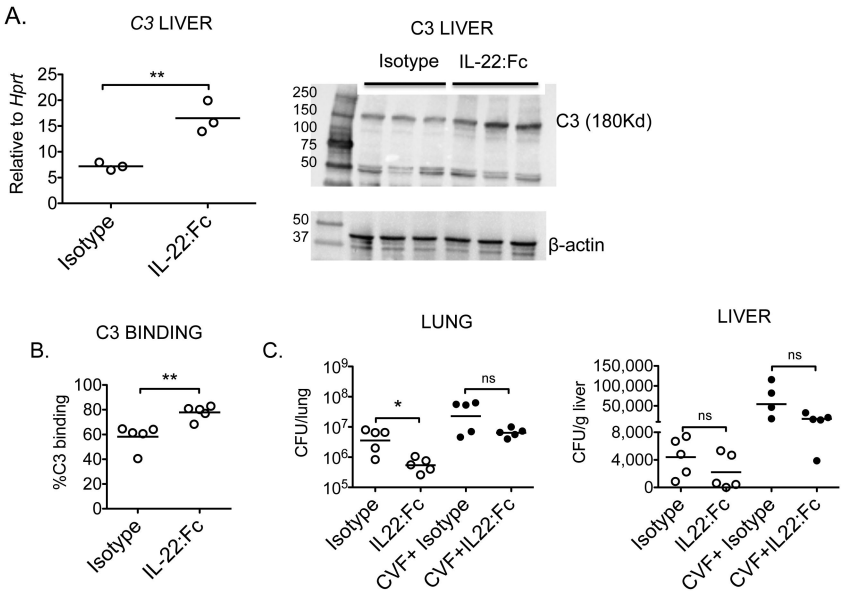

Figure 6. IL-22 increases serum opsonization capacity

A) *C3* gene expression in liver tissue from mice treated with Isotype or IL-22:Fc for 24 hours , **p< 0.01 (unpaired t-test). Protein expression by western blot of same liver homogenates. B) C3 binding assay. C3 deposition was measured on serum-opsonized Spn by flow cytometry. Data represent mean of the percentage of C3 positive bacteria exposed to serum from different mice: Isotype, IL-22:Fc or Spn treated (n = 5/group). C) C3 was depleted using cobra venom factor (CVF) (25 ug/mouse i.p) for 24 hours , followed by IL-22:Fc or isotype control pre treatment for 3 hours and Spn infection for 24 hours. * p <0.05, *** p<0.001, ns: p>0.05 (One-way ANOVA). Experiments were repeated twice.