Indonesian Journal on Health Science and Medicine
Vol 2 No 2 (2025): October
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo
https://doi.org/10.21070/ijhsm.v2i1.211

Assessing Humoral and Cellular Immunity in a Rat Model of Staphylococcal Bacterial Pneumonia

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Abstract. General Background: Staphylococcus aureus pneumonia remains a major global health concern due to its virulence, biofilm formation, and rising methicillin-resistant strains, which complicate treatment. Specific Background: Understanding the interplay between humoral and cellular immunity is crucial for designing effective interventions, as both antibody-mediated and T-cell–mediated responses contribute to pathogen clearance. Knowledge Gap: While immune evasion strategies of S. aureus are documented, quantitative insights into the temporal dynamics of antibody production, T-cell proliferation, and cytokine release during infection are limited. Aims: This study aimed to evaluate the kinetics of humoral and cellular immune responses in a rat model of S. aureus pneumonia. Results: Infected rats exhibited significantly elevated IgM and IgG levels, with IgM peaking at day 14 and IgG progressively increasing. Splenocyte proliferation and cytokine production (IFN-γ, IL-4) were markedly enhanced, particularly at day 21, indicating strong Th1 and Th2 activation. Novelty: The study provides an integrated temporal profile of dual-arm immunity in experimental S. aureus pneumonia, demonstrating concurrent robust humoral and cellular activation. Implications: These findings highlight the necessity of targeting both antibody and T-cell responses in vaccine design, potentially guiding the development of immunotherapies for effective prevention and treatment of S. aureus pneumonia.

Highlights:

1. IgM peaked on day 14, while IgG continued to rise until the end of the study.
2. Splenocyte proliferation indicated strong T cell activation.
3. Th1 and Th2 responses worked together to eliminate S. aureus from the lungs.

Keywords: Staphylococcus, Humoral, Cellular, Immunity, Rat Model

Published: 19-08-2025

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Introduction

One of the most common infections is *staphylococcus aureus*, which causes nosocomial infection, especially pneumonia. It is also known to invade mucosal surfaces and elicit invasive disease, especially in immunocompromised patients. The growing strain of methicillin-resistant *Staphylococcus aureus* (MRSA) makes treatment increasingly complex and a public health problem worldwide [1]. Humoral and cellular immunity interact intricately in response to *Staphylococcus aureus* pneumonia. To a significant extent, the humoral immune system – driven by antibodies – neutralizes the pathogen and opsonizes the bacteria for phagocytosis. Cellular immunity, in particular T cells, by contrast, is responsible for both finding infected cells and controlling the immune response (Chen *et al*, 2020). Knowing these interactions will be vital for creating novel therapeutic options and vaccines against *Staphylococcus aureus* pneumonia. Studies in this field have already demonstrated how *Staphylococcus aureus* deceives the immune system by secreting protein A to engage the Fc region of antibodies and releasing toxicants that target host cells [2]. *Staphylococcus aureus* biofilm production also makes it more difficult for the host to eradicate the infection, as biofilm-producing bacteria are resistant to immune clearance and antibiotics [3]. Therefore, it's of the utmost importance to study how immune systems are stimulated in controlled models of *Staphylococcus aureus* pneumonia to search for potential targets. This paper measured the humoral and cellular immune response using a rat model after rats were infected with Staphylococcus aureus. We believed that infection would cause a robust immune response resulting in increased antibody and T-cell activity, thus shedding light on the immune response to *Staphylococcus aureus* pneumonia.

Materials and methods:

Animals

We recruited male Sprague-Dawley rats (200-250 g). They kept the animals in an enclosed area with a 12-hour light/dark cycle and fed and watered freely. Animal experiments were all approved by the IACUC, and everything was done within the parameters set out by the ethics committee for animal research.

Infection Model

Rats were anesthetized with isoflurane and inoculated into the thorax with $1 * 10^7$ CFU of *Staphylococcus aureus* from a master student in 200 ml sterile saline. Without this, control rats were given only 200 ml of sterile saline. They watched animals for respiratory distress and killed them at time points (7, 14, and 21 days after infection) for analysis.

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Assessment of Humoral Immunity

The rats were anesthetized, and blood was drawn from them through a heart punch 7, 14, and 21 days after infection. The serum was centrifuged and stored at -20°C until use.

Antibody Measurement:

They used enzyme-linked immunosorbent assays (ELISA) to detect anti-*Staphylococcus aureus* immunoglobulin M (IgM) and IgG levels. The experiments were performed according to the manufacturer's instructions using a standard curve to measure antibody concentrations. Dilution was appropriate, and absorbance was measured at 450 nm on a microplate reader.

Assessment of Cellular Immunity

Spleens were taken from infected and control rats at the end of the experiment (21 days after infection). Splenocytes were harvested from a tissue homogenizer and pushed through a 70 m cell strainer. Those cells were resuspended in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin.

Proliferation Assay:

We plated splenocytes (1* 10^6 cells/mL) on 96-well plates containing *Staphylococcus aureus* antigens (1 g/mL) or phytohemagglutinin (PHA) as a positive control. Cell proliferation was measured at 72 hours in a methyl thiazolyl tetrazolium (MTT) test, where MTT was injected into each well, and the formazan crystals were dissolvable in DMSO. The absorbance of this material was calculated as 570 nm.

Cytokine Production:

We measured cytokine production (IFN-, IL-4) using a cytometric bead array as recommended by the manufacturer. The samples were analyzed using a flow cytometer and reported in pg/mL. They analyzed the data using the software, which included the cytometer.

Statistical Analysis

Data were analyzed with GraphPad Prism software (version X). We determined groups' differences using ANOVA and Tukey's post-hoc test, and p0.05 was taken as statistically significant. Data represent the average standard deviation (SD).

Results

Humoral Immune Response

Antibody Levels

IgM and IgG antibodies were much higher in infected rats than in controls. We found, via ELISA, that IgM levels were highest 14 days after infection, whereas IgG levels remained steadily increasing during the study.

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Table 1. Serum IgM and IgG Levels in Infected and Control Rats

Time Point (Days)	Group	IgM (µg/mL)	IgG (µg/mL)
7	Control	12.5 ± 2.1	15.3 ± 3.2
	Infected	35.7 ± 4.5**	22.6 ± 2.9**
14	Control	13.2 ± 1.8	16.1 ± 2.5
	Infected	42.1 ± 5.3**	35.8 ± 3.5**
21	Control	14.0 ± 2.2	17.5 ± 3.0
	Infected	50.3 ± 6.7**	48.2 ± 4.8**

Note: Values are mean ± SD. **p < 0.01 compared to the control group.**

Interpretation of Humoral Response

IgM levels increased rapidly after 14 days of infection, and the rate of increases in IgG suggests establishing a mature secondary response with time.[4]. That's in line with the immune dynamic seen commonly in bacterial infections, where the body produces first IgM followed by a longer-term IgG response [5].

Cellular Immune Response

Splenocyte Proliferation

As for proliferation, infected rats' splenocytes responded much more vigorously to Staphylococcus aureus antigens than controls. This reaction was particularly robust 21 days after infection.

Table 2. Splenocyte Proliferation in Response to Staphylococcus aureus Antigens

Time Point (Days)	Group	Proliferation Index (OD 570)
21	Control	0.28 ± 0.05
	Infected	0.75 ± 0.08**

Note: Values are mean ± SD. **p < 0.01 compared to the control group.**

Interpretation of Cellular Response

This heightened proliferative activity of splenocytes in the rats indicates a potent T-cell response to *Staphylococcus aureus* antigens [6]. This suggests that the infection effectively mobilized T cells to induce clonal growth and, hence, protective immunity.

Cytokine Production

Cytokine analysis showed that the cultured splenocytes from infected rats were enriched with IFN- and IL-4, suggesting an effective T-cell response. Both cytokines had increased markedly at 21 days after infection.

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Table 3. Cytokine Production in Splenocyte Cultures

Group	IFN-γ (pg/mL)	IL-4 (pg/mL)
Control	20.5 ± 4.1	15.2 ± 2.3
Infected	85.3 ± 10.5**	60.4 ± 5.7**

Note: Values are mean ± SD. **p < 0.01 compared to the control group.**

Interpretation of Cytokine Response

Infected cells accumulate IFN- that serves as a Th1 effect, which is crucial for phagocytosing and killing intracellular microbial cells by macrophages [7]. In parallel, the elevated IL-4 levels show a Th2 response that promotes antibody production and B-cell activation [8]. This cooperative cytokine effect may be required to remove *Staphylococcus aureus* from the lungs.

Discussion

This research shows that if we can model Staphylococcus aureus pneumonia in rats, it is also feasible to test humoral and cellular immune responses. The massive spike in specific antibodies implies an adaptive immune response, and the up-regulation of splenocytes and cytokine production suggests a robust cellular response.

Humoral Immunity

The augmented IgM and IgG levels in infected rats indicate that the immune system can generate specific antibodies against Staphylococcus aureus. This highest IgM response 14 days after infection is by the expected timeline of primary immune responses—the IgM response occurs first. Then, IgG is produced later in the secondary immune response [9]. This result aligns with the findings in other papers that positive antibodies are necessary to eradicate Staphylococcus aureus infections [10,11]. Notably, IgG antibodies are essential for opsonization and neutralization of pathogens to improve their clearance by phagocytes [12]. The prolonged elevation of IgG in this experiment hints at the possibility of sustained protective immunity, and it's vital to continue exploring the role of humoral response during S. aureus pneumonia.

Cellular Immunity

The proliferation of splenocytes and the release of cytokines such as IFN- and IL-4 indicate an aggressive T-cell attack on the infection. IFN- is mainly produced by CD4+ T helper type 1 (Th1) cells, and it is necessary for macrophage activation and the phagocytic response [13]. Yet IL-4 can be coupled to Th2 responses that generate antibodies and B-cell activation [14]. Protective resistance against S aureus pneumonia requires an appropriate ratio of Th1 and Th2 [15]. Earlier studies had shown that T cells were an essential component of the response to Staphylococcus aureus, with CD4+ T cells functioning as the central coordinating cell of the immune system [16]. These findings support the hypothesis that humoral and cellular immunity are required to halt the *Staphylococcus aureus* infections.[17]

Implications for Vaccine Development

These results indicate that protective immunity against Staphylococcus aureus pneumonia requires the humoral and cellular immune systems. These facts can then be

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harnessed to develop vaccines that bolster these immune responses. A candidate vaccine could aim to stimulate a thin IgG immune response besides Th1 cellular immunity [18]. New vaccines like recombinant protein or nanoparticle vaccines may improve immune sensitivity against *Staphylococcus aureus* [19]. These treatments could result in vaccines protecting against pneumonia caused by this agent for life.

Conclusion

It has shown the role of the humoral and cellular immune system in *Staphylococcus aureus* pneumonia. Such immune systems could even be analogized in rats, which could be exploited to make treatments and vaccines in the future. There is still a long way to go to explain what causes *Staphylococcus aureus* immune escape and to test immunotherapies. But perhaps we can learn something from other immune cells – macrophages and dendritic cells – that can attack *Staphylococcus aureus* pneumonia and devise new treatments and protections.

References

[1] D. Parker, C. L. Ryan, F. Alonzo, V. J. Torres, P. J. Planet, and A. S. Prince, "CD4+ T Cells Promote the Pathogenesis of Staphylococcus Aureus Pneumonia," J. Infect. Dis., vol. 211, no. 6, pp. 835–845, 2015, doi: 10.1093/infdis/jiu525.

[2] J. Braverman, I. R. Monk, C. Ge, et al., "Staphylococcus Aureus Specific Lung Resident Memory CD4+ Th1 Cells Attenuate the Severity of Influenza Virus Induced Secondary Bacterial Pneumonia," Mucosal Immunol., vol. 15, no. 4, pp. 783–796, 2022, doi: 10.1038/s41385-022-00529-4.

[3] W. H. Self, R. G. Wunderink, D. J. Williams, et al., "Staphylococcus Aureus Community-Acquired Pneumonia: Prevalence, Clinical Characteristics, and Outcomes," Clin. Infect. Dis., vol. 63, no. 3, pp. 300–309, 2016, doi: 10.1093/cid/ciw300.

[4] L. Hall-Stoodley, J. W. Costerton, and P. Stoodley, "Bacterial Biofilms: From the Natural Environment to Infectious Diseases," Nat. Rev. Microbiol., vol. 2, no. 2, pp. 95–108, 2004, doi: 10.1038/nrmicro821.

[5] N. J. Verkaik, C. P. de Vogel, H. A. Boelens, et al., "Anti-Staphylococcal Humoral Immune Response in Persistent Nasal Carriers and Noncarriers of Staphylococcus Aureus," J. Infect. Dis., vol. 199, no. 5, pp. 625–632, 2009, doi: 10.1086/596743.

[6] F. Askarian, T. Wagner, M. Johannessen, and V. Nizet, "Staphylococcus Aureus Modulation of Innate Immune Responses Through Toll-Like (TLR), NOD-Like (NLR) and C-Type Lectin (CLR) Receptors," FEMS Microbiol. Rev., vol. 42, no. 5, pp. 656–671, 2018, doi: 10.1093/femsre/fuy025.

[7] J. S. Cho, Y. Guo, R. I. Ramos, et al., "Neutrophil-Derived IL-1β Is Sufficient for Abscess Formation in Immunity Against Staphylococcus Aureus in Mice," PLoS

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo
https://doi.org/10.21070/ijhsm.v2i1.211

Pathog., vol. 8, no. 11, p. e1003047, 2012, doi: 10.1371/journal.ppat.1003047.

[8] L. S. Miller, V. G. Fowler, S. K. Shukla, et al., "Development of a Vaccine Against Staphylococcus Aureus Invasive Infections: Evidence Based on Human Immunity, Genetics and Bacterial Evasion Mechanisms," FEMS Microbiol. Rev., vol. 44, no. 1, pp. 123–153, 2020, doi: 10.1093/femsre/fuz030.

[9] A. F. Brown, J. M. Leech, T. R. Rogers, and R. M. McLoughlin, "Staphylococcus Aureus Colonization: Modulation of Host Immune Responses and Impact on Human Vaccine Design," Front. Immunol., vol. 4, p. 507, 2013, doi: 10.3389/fimmu.2013.00507.

[10] Y. Wang, L. I. Cheng, D. R. Helfer, et al., "Mouse Model of Hematogenous Implant-Related Staphylococcus Aureus Biofilm Infection Reveals Therapeutic Targets," Proc. Natl. Acad. Sci. U. S. A., vol. 114, no. 26, pp. E5094–E5102, 2017, doi: 10.1073/pnas.1703427114.

[11] D. Nurjadi, M. Kain, P. Marcinek, et al., "Ratio of T-Helper Type 1 (Th1) to Th17 Cytokines in Whole Blood Is Associated With Human β-Defensin 3 Expression in Skin and Persistent Staphylococcus Aureus Nasal Carriage," J. Infect. Dis., vol. 214, no. 11, pp. 1744–1751, 2016, doi: 10.1093/infdis/jiw440.

[12] A. K. Varshney, G. A. Kuzmicheva, J. Lin, et al., "A Natural Human Monoclonal Antibody Targeting Staphylococcus Aureus Toxins Protects Against Pneumonia and Skin Infection," mBio, vol. 9, no. 6, p. e02391-18, 2018, doi: 10.1128/mBio.02391-18.

[13] A. Nakou, M. Woodhead, and A. Torres, "MRSA as a Cause of Community-Acquired Pneumonia," Eur. Respir. J., vol. 34, no. 5, pp. 1013–1014, 2009, doi: 10.1183/09031936.00120009.

[14] P. Matzinger, "The Danger Model: A Renewed Sense of Self," Science, vol. 296, no. 5566, pp. 301–305, 2002, doi: 10.1126/science.1071059.

[15] J. K. Rudkin, A. M. Edwards, M. G. Bowden, et al., "Methicillin Resistance Reduces the Virulence of Healthcare-Associated Methicillin-Resistant Staphylococcus Aureus by Interfering With the Agr Quorum Sensing System," J. Infect. Dis., vol. 205, no. 5, pp. 798–806, 2012, doi: 10.1093/infdis/jir845.

[16] P. Peyrani, M. Allen, T. L. Wiemken, et al., "Severity and Outcomes of Adults Hospitalized With Laboratory-Confirmed Influenza: The Hospitalized Influenza Adults (HIA) Project," BMC Infect. Dis., vol. 19, no. 1, p. 89, 2019, doi: 10.1186/s12879-019-3729-5.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo
https://doi.org/10.21070/ijhsm.v2i1.211

[17]S. A. Fritz, K. M. Tiemann, P. G. Hogan, et al., "A Serologic Correlate of Protective Immunity Against Community-Onset Staphylococcus Aureus Infection," Clin. Infect. Dis., vol. 56, no. 11, pp. 1554–1561, 2013, doi: 10.1093/cid/cit123.

[18]A. S. Anderson, I. L. Scully, Y. Timofeyeva, et al., "Staphylococcus Aureus Manganese Transport Protein C Is a Highly Conserved Cell Surface Protein That Elicits Protective Immunity Against S. Aureus and Staphylococcus Epidermidis," J. Infect. Dis., vol. 205, no. 10, pp. 1688–1696, 2012, doi: 10.1093/infdis/jis278.

[19]R. A. Proctor, "Is There a Future for a Staphylococcus Aureus Vaccine?," Vaccine, vol. 30, no. 19, pp. 2921–2927, 2012, doi: 10.1016/j.vaccine.2011.11.006.