Project: Role of the mineralocorticoid receptor in inflammatory cells in bacterial infection

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Background and preliminary data:
Since the introduction of non-steroidal mineralocorticoid receptor (MR) antagonists and novel aldosterone synthase inhibitors, interest in the aldosterone system has significantly increased (Lother 2022). Recent studies have shown that the MR in myeloid and lymphocytic cells plays a pro-inflammatory and pro-fibrotic role as we recently summarized (van der Heijden 2022, Hengel 2022). Using a CD11cCre×$MR^{fl/fl}$ and a CD4Cre×$MR^{fl/fl}$ mouse models, we and others found that inactivation of the MR in $CD11c^+$ dendritic or $CD4^+$ T cells reduces blood pressure and end-organ damage in arterial hypertension (Hengel 2022).

Does the lack of the MR in dendritic cells worsen bacterial infection?
The MR acts as a pro-inflammatory factor in myeloid and lymphocytic cells (Hengel 2022). Deficiency of the MR in inflammatory cells has been shown to be beneficial in autoimmune and cardiovascular disease (van der Heijden 2022). However, there is a lack of data on the role of the MR in inflammatory cells during bacterial infections. If the MR promotes inflammation in dendritic and $CD4^+$ T cells, its inhibition could potentially be detrimental during infections, thus making its therapeutic targeting in humans impractical.
To investigate this crucial question, we will combine the CD11cCre×$MR^{fl/fl}$ and CD4Cre×$MR^{fl/fl}$ mouse models with two established infection models involving the kidney, spleen and liver. Systemic infection with *Listeria monocytogenes* induces a TH1 and $CD8^+$ T cell response, while infection with *Staphylococcus aureus* induces a TH17 cell response (Krebs 2020, Bertram 2023). We will examine both the innate and adaptive immune phases. Key parameters will include bacterial counts in infected organs, kidney function, and histological analysis. Additionally, we will study the $CD4^+$ and $CD8^+$ T cell response to the pathogens. Infection with ovalbumin-recombinant listeria will allow us to assess antigen-specific T cell responses (listeriolysin $O_{189\text{-}201}$ and $ovalbumin_{257\text{-}264}$).
We have observed increased salt concentration in the skin in hypertension and knockout of the MR in dendritic cells leads to a significant reduction in this concentration. A high-salt diet results in increased salt storage in the skin and enhanced infection defense against cutaneous infection (Hengel 2022). Therefore, we will investigate whether the MR knockout in dendritic cells leads to reduced defense in cutaneous staphylococcal infection.
For effective activation, T cells require recognition of peptide MHC complexes by their T cell receptor and co-signals provided by CD28 ligands on the antigen presenting cell. Following primary activation, T cells differentiate into specialized effector cells. These process is primarily controlled by soluble factors, such as cytokines, present in the local environment or secreted by the antigen presenting cells. We will examine whether MR signaling affects T cell activation and differentiation either directly or by altering the function of antigen presenting cells during bacterial infection.

Hypothesis: The MR in dendritic and lymphocytic cells plays an important role in control of bacterial infection.

Aims and Work Program:
1. Lack of the MR in dendritic cells or $CD4^+$ T cells aggravates bacterial infection.

2. Lack of the MR in $CD4^+$ T cells reduces the antigen-specific T cell response in listeria infection.
3. Overexpression of MR in dendritic or $CD4^+$ T cells shows increased resistance to infections.

Aim #1 Infection with *Listeria monocytogenes* and *Staphylococcus aureus*

Listeria: Intravenous infection with *Listeria monocytogenes* (strain LmOVA) will be performed in CD11cCre×$MR^{fl/fl}$ and CD4Cre×$MR^{fl/fl}$ mice. For studying the innate immune response, organs will be harvested 2 days post-infection, and for the adaptive immune response, on day 10. The bacterial dose will be 5×10^4 for early examination and 5×10^3 for late examination. On day 2, bacterial titers in the liver, spleen, and kidneys will be determined, and tissues will be collected for histology. Neutrophil and inflammatory monocyte mobilization will be analyzed using FACS. Bacterial titers in the liver, spleen, and kidneys will be quantified by plating serial dilutions of homogenized tissue, along with abscess counts in kidney tissue. The accumulation of myeloid cells and T cells in the spleen, kidneys, and liver will be determined using FACS and immunohistology (Krebs 2020).

Staphylococcus: Infection and analyses will be largely similar to those for *Listeria* infection. The intravenous dose of *Staphylococcus aureus* (strain SH1000) will be 1×10^7 bacteria (Bertram 2023). Parameters such as blood pressure, albuminuria, and GFR will be assessed. In the kidneys, histology and gene expression of markers of renal injury will be evaluated. For skin infection, an intradermal dose of 1×10^6 bacteria will be administered to the flank. In addition to the analyses already described, daily measurement of lesion size and histological examination of the infected skin area at the end of the experiment will be performed. Since no immunodominant peptides are available for *S. aureus*, T cells will be polyclonally stimulated to determine the general induction of TH1 and TH17 cells. Cells from the spleen, kidneys, and draining lymph nodes (in the case of skin infection) will be isolated and incubated with PMA and ionomycin in the presence of Brefeldin A, and cytokines will be measured by FACS.

Aim #2: Antigen-specific T cell response

For LmOVA, immunodominant epitopes in listeriolysin O for $CD4^+$ T cells and in ovalbumin for $CD8^+$ T cells are known, allowing the analysis of the strength and cytokine profile of specific T cell responses and thus the role of the MR in T cell activation and differentiation. Spleen cells will be incubated in vitro with $ovalbumin_{257-264}$ and listeriolysin $O_{189-201}$ peptides for 4 in the presence of Brefeldin A, and cytokines IFN-γ, TNF-α, and IL-17A, as well as CD40L expression in T cells, will be measured by FACS.

Aim #3: Overexpression of MR in myeloid and lymphocytic cells

Finally, we will examine whether mice with targeted overexpression of MR in dendritic cells or $CD4^+$ T cells exhibit increased resistance to infections caused by *Listeria* and *Staphylococcus aureus.*

In conclusion, we believe that this project will provide valuable new insights into the role of the MR in myeloid and lymphocytic cells during bacterial infections. By understanding the mechanisms underlying this process, we could pave the way for innovative treatments in cardiovascular and infectious diseases.

Project-related publications:

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Hengel F, Benitah JP, **Wenzel UO**. Mosaic theory revisited: inflammation and salt play a central role in arterial hypertension. Cell Mol Immunol. 2022, 19:561-576.

Krebs CF, Reimers D, Zhao Y, Paust HJ, Bartsch P, Nuñez S, Rosemblatt MV, Hellmig M, Kilian C, Borchers A, Enk LUB, Zinke M, Becker M, Schmid J, Klinge S, Wong MN, Puelles VG, Schmidt C, Bertram T, Stumpf N, Hoxha E, Meyer-Schwesinger C, Lindenmeyer MT, Cohen CD, Rink M, Kurts C, Franzenburg S, Koch-Nolte F, Turner JE, Riedel JH, Huber S, Gagliani N, Huber TB, Wiech T, Rohde H, Bono MR, Bonn S, Panzer U, **Mittrücker H-W**. Pathogen-induced tissue-resident memory T_H17 ($T_{RM}17$) cells amplify autoimmune kidney disease. Sci. Immunol. 2020, 5:eaba4163.

Lother A, Jaisser F, **Wenzel UO**. Emerging fields for therapeutic targeting of the aldosterone-MR signaling pathway. Br J Pharmacol. 2022, 179: 3099-3102.

van der Heijden CDCC, Bode M, Riksen NP, **Wenzel UO**. The role of the MR in immune cells in cardiovascular disease. Br J Pharmacol. 2022, 179:3135-3151.

Funding: Wenzel: WE 1688/19-3; Mittrücker: SFB1192 project A4, SFB1328 project A3.