In order of importance, list of ten best papers of the candidate, highlighting the important discoveries/contributions described in them briefly (Max 1.5 MB).

1. **Aging Cell** 22:2023:13838 [IF: 11]. Age-mediated gut microbiota dysbiosis promotes loss of tolerogenic potential in dendritic cells. Bashir H, Singh S, Singh RP, **Agrewala JN***, Kumar R*.

   Highlights of the manuscript. The old age-related loss of immune tolerance inflicts a person with a wide range of autoimmune and inflammatory diseases. Dendritic cells (DCs) are the sentinels of the immune system that maintain immune tolerance through cytokines and regulatory T-cell generation. Aging disturbs the microbial composition of the gut, causing immune system dysregulation. However, the vis-à-vis role of gut dysbiosis on DC tolerance remains highly elusive. Consequently, we studied the influence of aging on gut dysbiosis and its impact on the loss of DC tolerance. We show that DCs generated from either the aged (DC^{Old}) or gut-dysbiotic young ($DC^{Dysbiotic}$) but not young (DC^{Young}) mice exhibited loss of tolerance, as evidenced by their failure to optimally induce the generation of Tregs and control the overactivation of $CD4^+$ T cells. The mechanism deciphered for the loss of DC^{Old} and $DC^{Dysbiotic}$ tolerance was chiefly through the overactivation of NF-κB, impaired frequency of Tregs, upregulation in the level of pro-inflammatory molecules (IL-6, IL-1β, TNF-α, IL-12, IFN-γ), and decline in the anti-inflammatory moieties (IL-10, TGF-β, IL-4, IDO, arginase, NO, IRF-4, IRF-8, PDL1, BTLA4, ALDH2). Importantly, a significant decline in the frequency of the Lactobacillus genus was noticed in the gut. Replenishing the gut of old mice with the *Lactobacillus plantarum* reinvigorated the tolerogenic function of DCs through the rewiring of inflammatory and metabolic pathways. Thus, for the first time, we demonstrate the impact of age-related gut dysbiosis on the loss of DC tolerance. This finding may open avenues for therapeutic intervention for treating age-associated disorders with the *Lactobacillus plantarum*.

2. **J Biol Chem**. 2022 Oct 15:102596. [IF: 5.49]. *Mycobacterium tuberculosis* epitope entrapped in nanoparticles expressing TLR-2 ligand targeted to dendritic cells elicit protective immunity. Das DK, Zafar MA, Nanda S, Singh S, Lamba T, Bashir H, Singh P, Maurya SK, Nadeem S, Sehrawat A, Bhalla V*, **Agrewala JN***.

   Highlights of the manuscript. Novel vaccination strategies are crucial to efficiently control tuberculosis, as proposed by the World Health Organization under its flagship program "End TB Strategy." However, the emergence of drug-resistant strains of *Mycobacterium tuberculosis (Mtb*), particularly in those coinfected with HIV-AIDS, constitutes a major impediment to achieving this goal. We report here a novel vaccination strategy that involves synthesizing a formulation of an immunodominant peptide derived from the Acr1 protein of *Mtb*. This nanoformulation in addition

displayed on the surface a toll-like receptor-2 ligand to offer to target dendritic cells (DCs). Our results showed an efficient uptake of such a concoction by DCs in a predominantly toll-like receptor-2-dependent pathway. These DCs produced elevated levels of nitric oxide, proinflammatory cytokines interleukin-6, interleukin-12, and tumor necrosis factor-α, and upregulated the surface expression of major histocompatibility complex class II molecules as well as costimulatory molecules such as CD80 and CD86. Animals injected with such a vaccine mounted a significantly higher response of effector and memory Th1 cells and Th17 cells. Furthermore, we noticed a reduction in the bacterial load in the lungs of animals challenged with aerosolized live Mtb. Therefore, our findings indicated that the described vaccine triggered protective anti-*Mtb* immunity to control the tuberculosis infection.

3. **Cell Mol Life Sci.** 79:2022:567 [IF: 9.2]. *Mycobacterium tuberculosis* exploits MPT64 to generate myeloid-derived suppressor cells to evade the immune system. Singh S, Maurya SK, Aqdas M, Bashir H, Arora A, Bhalla V, **Agrewala JN***.

Highlights of the manuscript. *Mycobacterium tuberculosis (Mtb)* is a smart and successful pathogen since it can persist in the intimidating environment of the host by taming and tuning the immune system. *Mtb* releases MPT64 (Rv1980c) protein in high amounts in patients with active tuberculosis (TB). Consequently, we were curious to decipher the role of MPT64 on the differentiating dendritic cells (DCs) and its relation to evading the immune system. We observed that pre-exposure of differentiating DCs to MPT64 (DC^{MPT64}) transformed them into a phenotype of myeloid-derived suppressor cells (MDSCs). DC^{MPT64} expressed a high level of immunosuppressive molecules PD-L1, TIM-3, nitric oxide (NO), arginase 1, IDO-1, IL-10 and TGF-β, but inhibited the production of pro-inflammatory cytokines TNF-α, IL-6 and IL-12. DC^{MPT64} chemotaxis function was diminished due to the reduced expression of CCR7. DC^{MPT64} promoted the generation of regulatory T cells (Tregs) but inhibited the differentiation of Th1 cells and Th17 cells. Further, high lipid and methylglyoxal content, and reduced glucose consumption by DC^{MPT64}, rendered them metabolically quiescent and consequently, reduced DC^{MPT64} ability to phagocytose *Mtb* and provided a safer shelter for the intracellular survival of the mycobacterium. The mechanism identified in impairing the function of DC^{MPT64} was through the increased production and accumulation of methylglyoxal. Hence, for the first time, we demonstrate the novel role of MPT64 in promoting the generation of MDSCs to favor *Mtb* survival and escape its destruction by the immune system.

4. **Autophagy** 16:2020:1021 [IF: 16.01]. Induction of autophagy through Clec4e in combination with TLR-4: an innovative strategy to restrict the survival of *Mycobacterium tuberculosis.* Pahari S, Negi S, Aqdas M, Arnett E, Schlesinger LS, **Agrewala JN***.

Highlights of the manuscript. Host-directed therapies are gaining considerable impetus because of the emergence of drug-resistant strains of pathogens due to antibiotic therapy. Therefore, there is an urgent need to exploit alternative and novel strategies directed at host molecules to successfully restrict infections. The C-type lectin receptor CLEC4E and Toll-like receptor TLR4 expressed by host cells are among the first line of defense in encountering pathogens. Therefore, we exploited signaling of macrophages through CLEC4E in association with TLR4 agonists (C4.T4) to control the growth of *Mycobacterium tuberculosis (Mtb).* We observed significant improvement in host immunity and reduced bacterial load in the lungs of *Mtb*-infected mice and guinea pigs treated with C4.T4 agonists. Further, the intracellular killing of *Mtb* was achieved with a 10-fold lower dose of isoniazid or rifampicin in conjunction with C4.T4 than the drugs alone. C4.T4 activated MYD88, PtdIns3K, STAT1 and RELA/NFKB, increased lysosome biogenesis, decreased Il10 and Il4 gene expression and enhanced macroautophagy/autophagy. Macrophages from autophagy-deficient (atg5 knockout or Becn1 knockdown) mice showed elevated survival of *Mtb*. For the first time, this finding unveiled the novel role of CLEC4E in inducing autophagy through MYD88, which is required for the control of *Mtb* growth. This study suggests a unique immunotherapeutic approach involving CLEC4E in conjunction with TLR4 to restrict the survival of *Mtb* through autophagy.

5. **J Proteome Res**. [IF: 5.4]. 19:2020:4655. Deciphering the structural enigma of HLA class-II binding peptides for enhanced immunoinformatics-based prediction of vaccine epitopes. Chatterjee D, Priyadarshini P, Das DK, Mushtaq K, Singh B, **Agrewala JN***.

Highlights of the manuscript. Vaccines remain the most efficacious means to avoid and eliminate morbid diseases associated with high morbidity and mortality. Clinical trials indicate the gaining impetus of peptide vaccines against diseases for which an effective treatment still remains obscure. CD4 T-cell-based peptide vaccines involve immunization with antigenic determinants from pathogens or neoplastic cells that possess the ability to elicit a robust T helper cell response, which subsequently activates other arms of the immune system. The available *in silico* predictors of human leukocyte antigen II (HLA-II) binding peptides are sequence-based techniques, which ostensibly have balanced sensitivity and specificity. Structural analysis and understanding of the cognate peptide and HLA-II interactions are

essential to empirically derive a successful peptide vaccine. However, the availability of structure-based epitope prediction algorithms is inadequate compared with sequence-based prediction methods. In the present study an understanding of the structural aspects of HLA-II binders by analyzing the Protein Data Bank (PDB) complexes of pHLA-II has been provided. Furthermore, we mimicked the peptide exchange mechanism and demonstrated the structural implication of an acidic environment on HLA-II binders. Finally, demonstrated a structure-guided approach to decipher potential HLA-II binders within an antigenic protein. This strategy may accurately predict the peptide epitopes and thus aid in designing successful peptide vaccines.

6. **Eur J Immunol.** 16:2020:10 [IF: 6.8]. Intestinal microbiota disruption limits the isoniazid-mediated clearance of *Mycobacterium tuberculosis* in mice. Negi S, Pahari S, Bashir H, **Agrewala JN.**

   Highlights of the manuscript. Tuberculosis (TB) continues to remain a global threat due to the emergence of drug-resistant *Mycobacterium tuberculosis (Mtb)* strains and toxicity associated with TB drugs. The intestinal microbiota has been reported to affect the host response to immunotherapy and drugs. However, how it affects the potency of first-line TB drug isoniazid (INH) is largely unknown. Here, we examined the impact of gut microbial dysbiosis on INH efficiency in killing *Mtb*. In this study, we employed an *in vivo* mouse model, pretreated with broad-spectrum antibiotics (Abx) cocktail to disrupt their intestinal microbial population prior to *Mtb* infection and subsequent INH therapy. We demonstrated that microbiota disruption results in the impairment of INH-mediated *Mtb* clearance, and aggravated TB-associated tissue pathology. Further, it suppressed the innate immunity and reduced CD4 T-cell response against *Mtb*. Interestingly, a distinct shift of gut microbial profile was noted with an abundance of Enterococcus and a reduction of Lactobacillus and Bifidobacterium population. Our results show that the intestinal microbiota is a crucial determinant in efficacy of INH to kill *Mtb* and impacts the host immune response against infection. This work provides an intriguing insight into the potential links between host gut microbiota and potency of INH.

7. **J Infect Dis**. 211:2015: 486-96 [IF: 8.8]. Triggering through TLR-2 limits chronically stimulated Th1 cells from undergoing exhaustion. Chodisetti SB, Gowthaman U, Rai PK, Vidyarthi A, Khan N, **Agrewala JN***.

Highlights of the manuscript. Chronic infections result in T-cell exhaustion, a state of functional unresponsiveness. To control the infection, it is important to salvage the exhausted T cells. In this study, we delivered signals through Toll-like receptor 2 (TLR-2) to reinvigorate functionality in chronically activated T-helper type 1 (Th1) cells. This process significantly augmented the expression of T-bet, interferon γ, interleukin 2, and the anti-apoptotic molecule Bcl-2, whereas it dampened the display of the exhaustion markers programmed death receptor 1 (PD-1) and lymphocyte activation gene 3 (Lag-3). Additionally, TLR-2 signaling bolstered the ability of chronically stimulated Th1 cells to activate B cells. Finally, the results were substantiated by observing reduced lung pathology upon administration of TLR-2 agonist in the chronic infection model of tuberculosis. This study demonstrated the novel role of TLR-2 in rescuing chronically activated Th1 cells from undergoing exhaustion. This study will pave the way for targeting TLR-2 in developing therapeutic strategies to treat chronic diseases involving loss of Th1 cell function.

8. **J Biol Chem.** 289:2014:17515-28 [IF: 5.5]. Caerulomycin A enhances the TGF-β-Smad3 signalling by suppressing IFN-γ-STAT1 signalling to expand Tregs. Gurram RK, Kujur W, Maurya SK, **Agrewala JN*.**

Highlights of the manuscript. Cytokines play a very important role in the regulation of immune homeostasis. Regulatory T cells (Tregs) responsible for the generation of peripheral tolerance are under the tight regulation of the cytokine milieu. In this study, we report a novel role of a bipyridyl compound, Caerulomycin A (CaeA), in inducing the generation of regulatory T cells (Tregs). It was observed that CaeA substantially up-regulated the pool of Tregs, as evidenced by an increased frequency of CD4(+) Foxp3(+) cells. In addition, CaeA significantly suppressed the number of Th1 cells and Th17 cells, as supported by a decreased percentage of CD4(+)/IFN-γ(+) and CD4(+)/IL-17(+) cells, respectively. Furthermore, we established the mechanism and observed that CaeA interfered with IFN-γ-induced STAT1 signaling by augmenting SOCS1 expression. An increase in the TGF-β-mediated Smad3 activity was also noted. Furthermore, CaeA rescued Tregs from IFN-γ-induced inhibition. These results were corroborated by blocking Smad3 activity, which abolished the CaeA-facilitated generation of Tregs. This study indicates a novel role of CaeA in inducing the generation of Tregs. This finding suggests that CaeA has enough potential to be considered a potent future drug for the treatment of autoimmunity.

9. **J Infect Dis.** 209:2014:1436-45 [IF: 7.8]. Latency Associated Protein Acr1 Impairs Dendritic Cells Maturation and Functionality: A Possible Mechanism of Immune Evasion by *Mycobacterium tuberculosis.* Siddiqui KF, Amir M, Gurram RK, Khan N, Arora A, K Rajagopal, **Agrewala JN***.

Highlights of the manuscript. *Mycobacterium tuberculosis (M. tuberculosis)* in latently infected individuals survives and thwarts the attempts of eradication by the immune system. During latency, Acr1 protein is predominantly expressed by *M. tuberculosis*. However, whether *M. tuberculosis* exploits its Acr1 in impairing the host immunity remains widely unexplored. In this study, we have investigated the role of Acr1 in influencing the differentiation and function of dendritic cells (DCs), which play a cardinal role in innate and adaptive immunity. Therefore, for the first time, we have revealed a novel mechanism of mycobacterial Acr1 in inhibiting the maturation and differentiation of DCs by inducing tolerogenic phenotype by modulating the expression of PD-L1; Tim-3; indoleamine 2, 3-dioxygenase (IDO); and interleukin 10. Furthermore, Acr1 interferes with the differentiation of DCs by targeting STAT-6 and STAT-3 pathways. Continuous activation of STAT-3 inhibited the translocation of NF-κB in Acr1-treated DCs. Furthermore, Acr1 augmented the induction of regulatory T cells. These DCs displayed a decline in their antigen uptake capacity and reduced ability to help T cells. Interestingly, *M. tuberculosis* exhibited better survival in Acr1-treated DCs. Thus, this study for the first time provides a crucial insight into a strategy adopted by *M. tuberculosis* to survive in the host by impairing the function of DCs.

10. **J Infect Dis**. 204:2011:1328-38. [IF: 7.8]. The promiscuous peptide of 16 kDa antigen linked to Pam2Cys protects against *M. tuberculosis* by evoking enduring memory T cells response. Gowthaman U, Singh V, Zeng W, Jain S, Siddiqui KF, Chodisetti SB, Gurram RK, Parihar P, Gupta P, Gupta UD, Jackson DC, **Agrewala JN***.

Highlights of the manuscript. One of the main reasons considered for BCG failure in tuberculosis-endemic areas is impediment by environmental mycobacteria in its processing and generation of memory T-cell response. To overcome this problem, we developed a unique lipopeptide (L91) by linking the promiscuous peptide (sequence 91-110) of 16 kDa antigen of *Mycobacterium tuberculosis* to TLR-2 agonist Pam2Cys. L91 does not require extensive antigen processing and generates an enduring Th1 memory response. This is evidenced by the fact that L91 significantly improved the activation, proliferation, and generation of protective T cells. Furthermore, L91 surmounts the barrier of major histocompatibility complex

polymorphism and induces better protection than BCG. This peptide has self-adjuvanting properties and activates dendritic cells. Importantly, L91 activates T cells isolated from purified protein derivative-positive healthy volunteers that responded weakly to the free peptide (F91). In essence, L91 can be a potent future vaccine candidate against tuberculosis.