Dual Monitoring of Impedance-Based Barrier Function and NF-κB Reporter Signaling in a Caco-2 Intestinal Inflammation Model

M. Schaffer and J. Heilmann

Department of Pharmaceutical Biology, University of Regensburg, Regensburg (Germany)
michael.schaffer@ur.de

Chronic inflammatory bowel disease (CIBD) is associated with factors such as the microbiome, genetics, and environmental influences. However, a dysregulated immune response, including cytokine signaling, and impaired intestinal barrier function are especially contributing to disease pathogenesis [1]. Besides interleukin-1β (IL-1β) and interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) is particularly known for its critical role in regulating intestinal inflammation and is therefore a key target for biological therapies in CIBD [2]. However, TNF-α signaling in intestinal epithelial cells (IECs) is pleiotropic, contributing not only to pathological processes but also to the maintenance of intestinal homeostasis [3,4].
The transcription factor NF-κB is a key transducer of TNF-α and IL-1β signaling [5]. To monitor its activation over time, we developed a stable Caco-2 reporter cell line expressing a secreted luciferase. In combination with impedance spectroscopy, this system enables simultaneous monitoring of NF-κB activity and barrier function during cytokine-induced inflammation in a differentiated Caco-2 intestinal model (see Figure 1). Interestingly, IL-1β did not influence transepithelial electrical resistance (TEER), but increased NF-κB activation. While TNF-α alone had minimal impact on IEC barrier function, IFN-γ led to an initial TEER increase. Co-treatment revealed synergistic TEER breakdown after the initial increase. As NF-κB activity was not significantly altered by co-stimulation with IFN-γ, additional signaling pathways might be involved. Furthermore, IFN-γ stimulation was direction-independent, whereas TNF-α stimulation showed higher sensitivity of the basolateral membrane.
Our model provides valuable insights into cytokine-mediated responses of IECs, integrating intracellular signaling with functional readouts. A better understanding of these mechanisms may contribute to the development of more effective targeted therapies for CIBD.

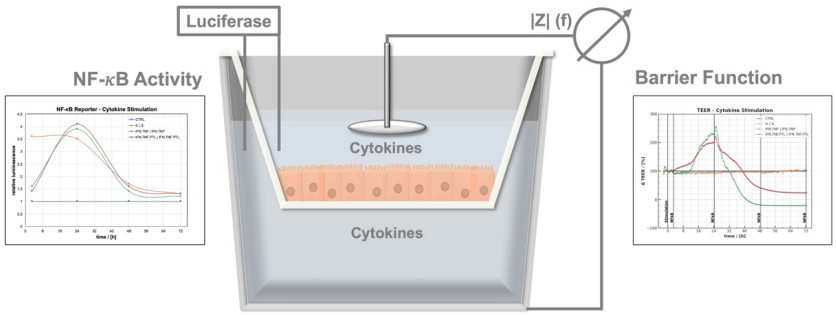

Figure 1: Dual monitoring of NF-kB activation by Caco-2 reporter cell line and impedance-based barrier function during cytokine-induced inflammation

References:

[1] J. Jang and S. Jeong, *BioChip Journal* 17 (2023) 403–430.
[2] M. Friedrich; M. Pohin and F. Powrie, *Immunity* 50 (2019) 992–1006.
[3] M. E. Delgado and T. Brunner, *Genes and Immunity* 20 (2019) 609–626.
[4] P. E. Dubé; S. Punit and D. B. Polk, *American Journal of Physiology-Gastrointestinal and Liver Physiology* 308 (2015) G161–G170.
[5] Q. Guo; Y. Jin; X. Chen; X. Ye; X. Shen; M. Lin; C. Zeng; T. Zhou and J. Zhang, *Signal Transduction and Targeted Therapy* 9 (2024) 53.