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INSIGHTS

Send it, receive it, quick erase it: A mouse model to decipher chemokine communication

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A method to precisely determine which cells respond to chemokines in vivo is currently lacking. A novel class of dual fluorescence reporter mice could help identify cells that produce and/or sense a given chemokine in vitro and in vivo (Rodrigo et al. 2024. *J. Exp. Med.* https://doi.org/10.1084/jem.20231814).

Chemokines (or chemotactic cytokines) are a large family of small molecules that are critically important for lymphocyte migration during infection and inflammation and thus steer the initial phases of the immune response. Apart from orchestrating lymphocyte migration, chemokines can also regulate the proliferation, function, and survival of leukocytes and are thus critical for the development and homeostasis of the immune system. Moreover, they can influence the function of structural cell types such as epithelial and endothelial cells (Hughes and Nibbs, 2018). Despite their central role in shaping both innate and adaptive immune responses, few tools exist to identify cells that respond to chemokines in an unbiased manner. Most of what is known about functional chemokine networks today is based on indirect measurements (e.g., single-cell RNA sequencing, proteomics, cell supernatant ELISA, or mathematical algorithms that are not chemokine specific and do not take dosage and cell positioning in vivo into account) (Armingol et al., 2021). The study of chemokines is further impeded by the complexity of chemokine networks, which includes redundancy and promiscuity at the level of both the chemokines as well as their receptors. In addition, chemokines are notorious for their capacity to oligomerize with each other, thereby modifying their biological activity (von Hundelshausen et al., 2017). CCL3, for example, has been shown to interact with CCL4 and signal through CCR1, CCR4, and CCR5 (Colobran et al., 2007). Due to this complexity, studying chemokines in vitro can lead to biased results, and an unbiased in vivo approach would add great value to the field.

In this issue of *JEM*, Rodrigo et al. (2024) report the generation of a novel class of dual fluorescence reporter mice to bridge this gap. The described CCL3-EASER (ErAse, SEnd, Receive) mouse model enables the evaluation of transcriptional and translational regulation of chemokine production in vivo, the identification and inducible ablation of cells producing CCL3 transcripts, and the detection of CCL3-sensing cells (see figure). They demonstrate the importance of this system by showing that natural killer (NK) cells are the first and primary source of CCL3 during the early phase of murine cytomegalovirus (MCMV) infection, rather than macrophages as commonly accepted (Menten et al., 2002; Salazar-Mather et al., 2002). Moreover, the authors were able to show that CCL3 mainly acts in an auto-/paracrine manner, stimulating NK cell cross-talk and their activity against MCMV in the early phase of infection. The EASER system also led to the discovery that type I IFN induces organ-specific post-transcriptional regulation of CCL3 during MCMV infection, which suggests mechanisms of cell type–specific post-transcriptional regulation of chemokine production in vivo. This point is well taken given that most chemokine networks predicted in silico from single-cell RNA sequencing atlases in mouse and human tissues have not been validated at the protein level (Hildreth et al., 2021). Thus, additional chemokine EASER mice will be necessary to systematically test hypotheses generated from these datasets and challenge current dogmas in chemokine biology.

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The flexibility of the EASER system supports additional studies of responder cell networks in vivo. Indeed, the construct can be adapted to encode other chemokines, cytokines, and even hormones, as long as the ligand-receptor complexes are endocytosed by the responder cell following signaling. This technical advance will allow validation of in silico–predicted cell–cell communication networks (Hildreth et al.,

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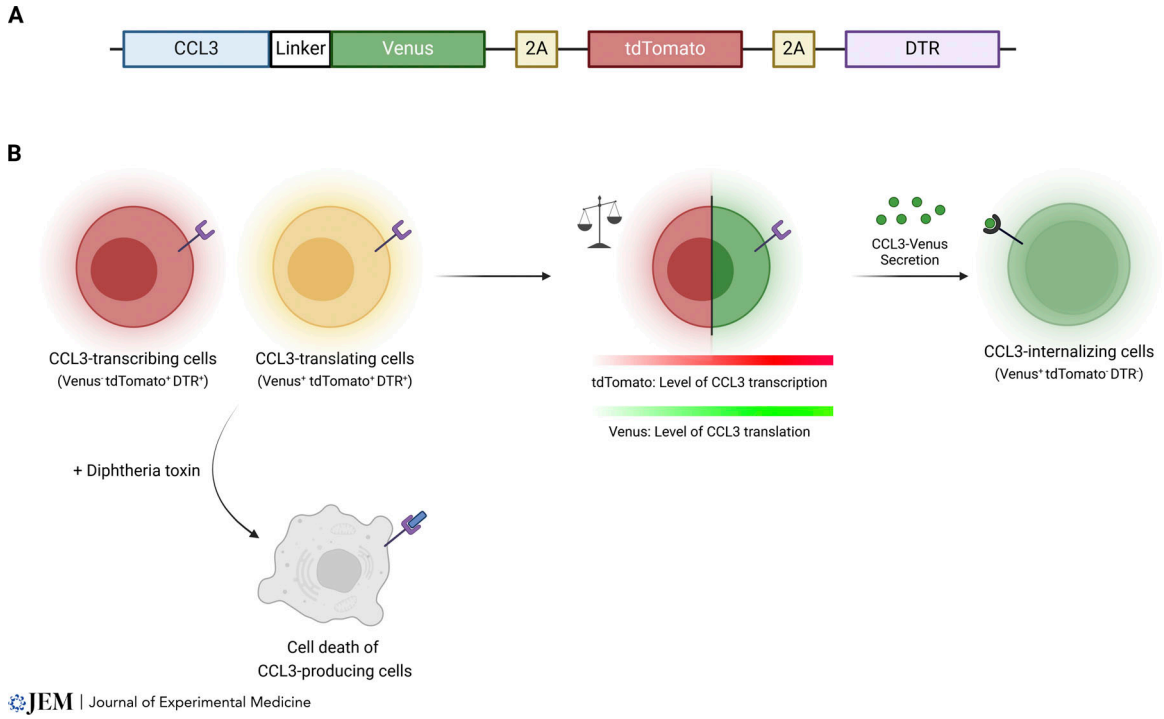

Principle of the EASER construct. (A) The EASER knock-in construct. (B) Upon transcription of CCL3, cells will start expressing tdTomato and the DTR receptor, while Venus is expressed upon translation of the CCL3 transcript. Correlating tdTomato and Venus levels can be used to approximate the translational activity on a single-cell level. Identification of responder cells relies on the internalization of the Venus-labeled CCL3 upon receptor-mediated signaling. Finally, CCL3-expressing cells can be selectively ablated by adding diphtheria toxin. Figure created in https://BioRender.com.

2023) as well as define tissue-resident and systemic signaling niches in the context of health and disease states. Inversely, the EASER mice system could also be used to unbiasedly identify cell types that respond to individual chemokines in vivo through the sorting of tdTomato-Venus⁺ cells and subsequent profiling by single-cell RNA sequencing. The EASER construct could also be modified by introducing a lox-stop-lox cassette, allowing for a cell type–specific or tamoxifen-induced tracking of communication networks in vivo. Chemokines also play an important role in chronic inflammatory diseases (reviewed in White et al., 2013) and the tumor microenvironment (reviewed in Nagarsheth et al., 2017). However, up until now, therapeutic targeting of chemokine signaling pathways has focused on a single chemokine or receptor at a time and has yielded unsatisfactory clinical results. This might be due to a lack of understanding of the complex chemokine network that hinges on redundancy, although inappropriate target selection and inadequate dosing have also been proposed as possible hurdles (Schall and Proudfoot, 2011). It has been hypothesized that chemokine biology might rather be highly specific instead of redundant and that each combination of chemokine oligomerization or receptor interaction triggers a slightly different response (Dyer, 2020). Either way, the EASER mice system could help elucidate the chemokine interactome in mice and thereby increase our understanding of multivariant therapeutical interventions in chemokine signaling in a wide range of disease models.

While the EASER system represents an exciting advance in the field, there are several limitations of the model that should be discussed. First, the detection of responding cell types relies on the uptake of the fluorescently labeled fusion proteins, excluding secreted proteins that can signal without being endocytosed by the responder cell. It is also crucial to distinguish between tdTomato⁻Venus⁺ cells that could result from phagocytosis/endocytosis versus receptor-mediated uptake followed by signaling. Indeed, it has been shown that macrophages can phagocytose activated NK cells, which may lead to false-positive CCL3 responder macrophages in vivo (Li et al., 2024). Through a transwell experiment with WT NK cells, the authors were able to show that Venus-positive responder cells displayed a typical chemotaxis response, indicating that they likely responded to CCL3 rather than only acquiring Venus fluorescence. However, this readout is not possible in vivo, and putative chemokine responder cells will need to be identified with caution, perhaps with the exclusion of highly phagocytic or endocytic cells. Another point to consider is that selectively deleting CCL3-producing cells using the diphtheria toxin receptor (DTR) can result in unintended cell death due to off-target effects. The reason for this is that a single DTR molecule on a cell can make it vulnerable to death when exposed to diphtheria toxin (Saito et al., 2001), while such a low level of transcription does not lead to detectable levels of tdTomato. Consequently, the toxin treatment might kill more cells than intended, and since these cells may not be detectable as tdTomato positive, the extent and nature of this off-target killing may limit the use of inducible depletion to test the role of chemokine-producing cells in homeostasis or disease contexts. Despite these caveats, the newly reported EASER mouse represents a powerful

tool to further unravel complex chemokine networks in vivo. The importance of this is underscored by the central role of chemokines in steering immune responses upon infection, in chronic inflammatory diseases, and in the tumor environment. Therefore, a better understanding of chemokine communication networks may pave the way for novel therapeutic strategies for various diseases.

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