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INSIGHTS

Macro-clusters: CD301b⁺ DCs prime Th2 responses

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In this issue of *JEM*, Lyons-Cohen et al. (https://doi.org/10.1084/jem.20231282) reveal that lymph node macro-clusters provide a spatial niche where CD301b⁺ cDC2s and CD4⁺ T cells interact. These integrin-mediated cellular hubs promote enhanced co-stimulation and cytokine signaling to drive Th2 differentiation.

Dynamic migration and cellular interaction is a key characteristic of the immune system. How precise immune interactions direct specific immune outcomes is not fully understood. In this issue of *JEM*, Lyons-Cohen and colleagues shed light on spatiotemporal lymph node interactions that facilitate T helper 2 (Th2) priming. This study revealed that adhesion-dependent CD301b⁺ conventional type 2 dendritic cells (cDC2s) and CD4⁺ T cells form large clusters in both allergic and helminth Th2 responses. This work has implication for how specific immune outcomes can be directed to either promote pathogen clearance or inhibit pathogenic T cell formation.

A successful immune response involves the interplay of innate and adaptive immune responses resulting in a heterogeneous CD4⁺ Th cell pool that is honed to specific classes of pathogens (O'Shea and Paul, 2010). Differentiation of each Th subset requires a specific cytokine milieu that is influenced by the pathogen type (Hilligan and Ronchese, 2020). Infection with virus or intracellular bacteria as well as cancer result in an enriched IL-12 microenvironment that favors the generation of IFNγ-secreting Th1 cells. Th2 cells are formed during helminth infection or upon contact with allergens and produce IL-4, IL-5, and IL-13. Th17 cells are induced by IL-6, TGFβ, IL-1β, and IL-23 and are essential for the clearance of extracellular bacteria and fungi by secreting IL-17. To promote germinal center formation, follicular T helper (Tfh) cells are generated with the aid of IL-6 and inducible co-stimulator (ICOS). These directed immune responses can overlay with co-opted pathogenic responses, such as Th2 for allergy and asthma, and Th1 and Th17 with autoimmunity. To counteract an excessive immune response and prevent autoimmunity, CD4⁺ T cells can further develop into inducible regulatory T cells. Importantly, this theoretical model is not rigid: some T cell clones develop a dynamic plasticity that can result in simultaneous development of multiple Th subsets (Becattini et al., 2015). While generally immune responses are tailored via the generation of specialized Th effector populations, there is a certain heterogeneity within Th populations (O'Shea and Paul, 2010). This provides the immune system flexibility to react with the optimal and most efficient immune response and to adapt to overshooting immune processes resulting in autoimmunity or allergy. Lyons-Cohen and colleagues address a fundamental question of how this specificity is generated, with a focus on the spatiotemporal aspect of Th2 differentiation within antigen-draining lymph nodes.

The decisions between alternate Th effector fates are influenced by antigen route, affinity, load, and activation of pathogen-associated molecular pattern molecules (PAMPs). However, the precise DC–T cell interactions that individually drive Th diversification remain unclear. CD4⁺ differentiation is a multistep process where CD4⁺ T cells engage in multiple serial DC interactions, which sum to direct Th fate (Duckworth and Groom, 2021). Previous work from Gerner and colleagues has highlighted the precise inflammation-induced positioning of DCs within the lymph node (Leal et al., 2021). Lyons-Cohen et al. (2024) build on this work to show that migratory cDC2s, marked by CD301b⁺, localize to the lymph node T–B cell border after cutaneous administration of antigen in complex with the allergen papain. Here, CD301b⁺ facilitate CD4⁺ T cell interactions, which they named "macro-clusters." These clusters of antigen-specific CD4⁺ T cells are characterized by high proliferation, GATA3 and IRF4 expression, and IL-4 production, which together signify nascent Th2 differentiation. Proximity analysis defined CD301b⁺ DCs within this neighborhood, while lymph node–resident cDC2s remain in the paracortex. Similar macro-clusters formation was identified early during Th2 differentiation

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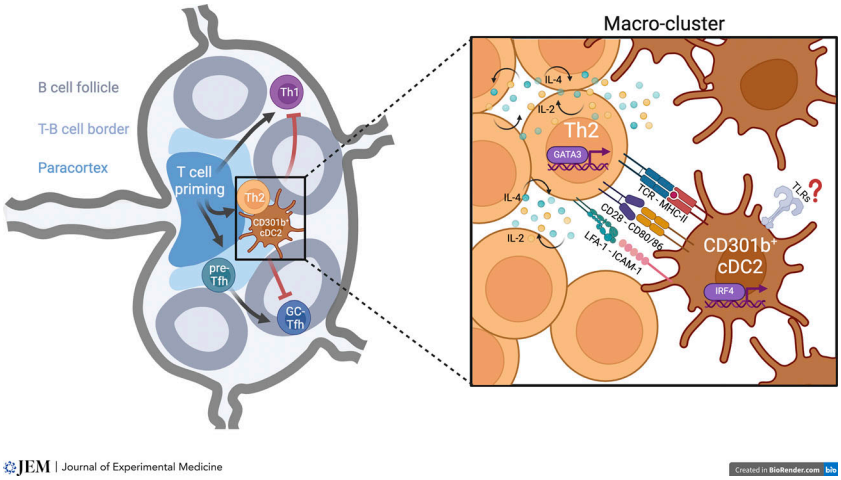

Spatiotemporal control of CD4⁺ helper differentiation in the lymph node. Upon pathogen contact, a heterogenous CD4⁺ Th pool tailored toward a specific type of pathogen is initiated by T cell priming in the paracortex (blue) in the center of the lymph node. Following a type I stimulus, activated T cells migrate from the paracortex into the peripheral medulla via an CXCL9/10 axis where they differentiate into Th1 cells. An early differentiation stage of Tfh cells (pre-Tfh) are formed in the T–B cell border (light blue) in the presence of IL-6 and ICOS. They then re-locate to the B cell follicle (gray) to develop into mature germinal center (GC) Tfh. Lyons-Cohen and colleagues describe the formation of macro-clusters in the T–B cell border of the lymph node upon helminth infection and allergen contact. In these macro-clusters (enlarged in the insert), CD301b⁺ cDC2s engage with activated CD4⁺ T cells that are mediated by LFA-1–ICAM-1 interaction. Upon antigen-specific TCR–MHC-II binding, expression of co-stimulatory molecules such as CD80/CD86 on CD301b⁺ cDC2s is significantly enhanced, which increases T cell CD28 co-stimulation. Macro-clusters are characterized by induction of the Th2 lineage driving transcription factors GATA3 and IRF4 and the production of the cytokines IL-4 (cyan) and IL-2 (yellow), which further amplify and direct T cell differentiation. The involvement of PAMP or TLRs in this process remains an open question. In summary, macro-clusters provide spatial proximity of CD4⁺ T cells and CD301b⁺ cDC2s as well as cytokine signaling hubs that create an optimal environment for Th2 development in the lymph node and comes at the expense of Th1 and Tfh generation.

following skin infection with the helminth, *Nippostrongylus brasiliensis*. These observations contrast the cellular contacts identified for other Th fates. In type I inflammation, spatial re-organization of DCs and T cells occurs in the lymph node to optimize interaction and facilitate efficient T cell priming and Th1 differentiation. Resident cDCs migrate from the periphery via CCR7 to the T cell zone in the paracortex to induce priming of naïve T cells. Myeloid cells such as inflammatory monocytes cluster in the lymph node within the first hours of an immune reaction and assist DCs by providing Th1-driving cytokines like IL-12 (Leal et al., 2021). Within 24 h after immunization, CXCR3-expressing T cells have been shown to migrate from the T cell zone in the center of the lymph node into the periphery via an CXCL9/10 axis. In the interfollicular and medullary regions, T cells interact with CXCL10⁺ DCs and develop into Th1 cells (Groom et al., 2012). Th2-initiating macro-clusters were first observed on day 2–3 following Th2-biased immunization; thus, the timing when Th1 and Th2 differentiation processes initially separate is currently unclear (see figure). Still, the work of Lyons-Cohen et al. (2024) builds on previous studies that demonstrate CD301b⁺ cDC2s favor Th2 responses at the expense of other CD4⁺ Th fates. Indeed, CD301b⁺ cDC2s promote differentiation toward Th2 with a reciprocal loss of both Th1 and Th17 cells and negatively regulate humoral immunity by preventing Tfh generation (Kumamoto et al., 2016; Tatsumi et al., 2021). Combined, these works highlight the role of CD301b⁺ DCs as key regulators of how pathogen-specific immune responses are directed.

At the outset, the authors highlight the universal requirement for macro-clusters with different Th2 stimulus: using OVA-papain vaccination and helminth infection. Intriguingly, they demonstrate that the route of administration is critically important for the establishment of Th2-inducing cell clusters. While administration of papain or alum into the ear pinnae led to extensive formation of macro-clusters in auricular draining lymph nodes, immunization into the footpad resulted in significantly reduced Th2 responses in the draining brachial lymph nodes. Despite extensive proliferation and activation of antigen-specific T cells, in this setting, CD4⁺ T cells were distributed throughout the brachial lymph node and accumulated in small numbers in the T cell zone. This was in contrast to other dermal routes, which led to extensive macro-cluster formation within the brachial lymph node. Interestingly, induction of type I inflammation resulted in consistent Th1 generation, independent of the administration site. These findings provided the authors the opportunity to understand the mechanisms that underlie macro-cluster–directed Th2 responses. Based on bulk RNA sequencing of antigen-bearing CD103b⁺ cDC2s of auricular and brachial lymph nodes, they hypothesize that these differences might be due to the differential presence of costimulatory molecules. Indeed, increased expression of the co-stimulatory molecules CD80 and CD86 on CD103b⁺ cDC2s from auricular lymph nodes was associated with enhanced TCR signaling, T cell activation, and IL-2 and IL-4 production. Increased co-stimulation also directly correlated with the expression of Th2-characteristic transcription factors GATA3 and IRF4. As delayed blockade of CD28 resulted in decreased formation of macro-clusters, the authors suggest a link between enhanced co-stimulation and an IL-2 and IL-4 cytokine reservoir that potentiates Th2 differentiation (figure insert). Supporting this, macro-clusters were associated with enhanced Th2-driving cytokines and increased T cell signaling. The importance of increased co-stimulation is in contrast to previous studies that showed that downmodulation of CD86 and MHC-II promotes Th2 differentiation while a strong TCR stimulus with prolonged T–DC bindings favors Th1 over Th2 development (Castellanos et al., 2021; van Panhuys et al., 2014). These discrepancies likely stem from differences in experimental systems and timepoints of analysis.

Previous work has demonstrated that dermal CD301b⁺ cDCs are essential for Th2 differentiation as they transport the antigen from the site of immunization into the draining lymph node for display and assist the accumulation of T cells (Kumamoto et al., 2013; Perner et al., 2020; Tatsumi et al., 2021). It is not clear if CD301b⁺ DCs

exhibit a distinct set of PAMPs and Toll-like receptors (TLRs) to mediate Th2 differentiation (Iwasaki and Medzhitov, 2004). TLR stimulation promotes DC maturation associated with the expression of costimulatory molecules such as CD80 and CD86, leading to the upregulation of CCR7 on DCs that influences lymph node migration. Rather than using direct classical PAMP and TLR signaling, dermal CD301b$^+$ DCs may instead be triggered by sensory neurons to provide cell migration signals (Perner et al., 2020). If this pathway impacts the formation of route-specific macro-cluster formation is unknown. Further, it may be that CD301b$^+$ DCs act primarily as agents of adhesion to promote cell clustering and ensure extended priming. Lyons-Cohen and colleagues used LFA-1 blocking to show that macro-clusters are destroyed in the absence of cell adhesion. Although it is likely that T–DC interactions are optimized by ICAM–LFA-1 binding, it is not clear if LFA-1 blocking instead affects interactions between newly activated T cells themselves. However, this result phenocopied the results with IRF4-deficient DCs, establishing the essential role of CD301b$^+$ cells for macro-cluster formation. Consistent with the concept that CD301b$^+$ DCs promote cell adhesion, previous studies have established that CD301b$^+$ DCs increase the dwell time of naïve CD4$^+$ T cells in the draining lymph node by antigen-independent MHC-II–TCR interactions (Tatsumi et al., 2021).

In summary, Lyons-Cohen and colleagues describe macro-clusters as cytokine signaling hubs where T cells are able to support each other's differentiation via the production of IL-2 and IL-4 to initiate Th2 differentiation. While evidence of macro-cluster formation in human Th2 responses remains to be seen, this study reveals tangible pathways that may be exploited to either drive protective Th2 or inhibit pathogenic Th2 responses in the future.

References

Becattini, S., et al. 2015. *Science*. https://doi.org/10.1126/science.1260668

Castellanos, C.A., et al. 2021. *Sci. Immunol.* https://doi.org/10.1126/sciimmunol.abh0707

Duckworth, B.C., and J.R. Groom. 2021. *Immunol. Rev.* https://doi.org/10.1111/imr.12945

Groom, J.R., et al. 2012. *Immunity*. https://doi.org/10.1016/j.immuni.2012.08.016

Hilligan, K.L., and F. Ronchese. 2020. *Cell. Mol. Immunol.* https://doi.org/10.1038/s41423-020-0465-0

Iwasaki, A., and R. Medzhitov. 2004. *Nat. Immunol.* https://doi.org/10.1038/ni1112

Kumamoto, Y., et al. 2016. *Elife*. https://doi.org/10.7554/elife.17979

Kumamoto, Y., et al. 2013. *Immunity*. https://doi.org/10.1016/j.immuni.2013.08.029

Leal, J.M., et al. 2021. *Sci. Immunol.* https://doi.org/10.1126/sciimmunol.abb9435

Lyons-Cohen, M., et al. 2024. *J. Exp. Med.* https://doi.org/10.1084/jem.20231282

O'Shea, J.J., and W.E. Paul. 2010. *Science*. https://doi.org/10.1126/science.1178334

Perner, C., et al. 2020. *Immunity*. https://doi.org/10.1016/j.immuni.2020.10.001

Tatsumi, N., et al. 2021. *Sci. Immunol.* https://doi.org/10.1126/sciimmunol.abg0336

van Panhuys, N., et al. 2014. *Immunity*. https://doi.org/10.1016/j.immuni.2014.06.003