COMMENT

Aiolos: a molecular guardian of type 2 innate immune cell residency and response

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Recent findings by Felton et al. and Qiu et al. unveil the importance of the transcription factor Aiolos for tissue-resident eosinophils and group 2 innate lymphoid cells (ILC2s). The authors find that Aiolos regulates the transcriptomic profile and chromatin landscape, both of which are essential for eosinophil and ILC2 localisation in tissues, as well as their function. These studies place Aiolos as a molecular guardian that regulates tissue-resident type 2 innate cells.

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One of the key attributes of the immune system is the rapid and effective response to infection by innate and adaptive immune cells. Traditionally, it was thought that immune cells recirculate throughout the body, transiting between the blood and lymphoid organs, and only migrating to effector sites in tissues upon exposure to danger signals. However, it is now clear that there are non-circulating, tissue-resident innate and adaptive immune cells that are critical for the initiation of immune responses and provide a first line of defence against pathogens[1]. However, the precise molecular pathways that control tissue migration, localisation and function remain unclear. In this issue of *Mucosal Immunology*, two papers identify a role for the transcription factor Aiolos in the tissue-specific localisation and function of eosinophils and type 2 innate lymphoid cells (ILC2s). Together, these studies place Aiolos as a central regulator of tissue-resident type 2 innate immune cells (Fig. 1).

Aiolos is a member of the Ikaros Zinc Finger (IKZF) family of DNA-binding proteins and is encoded by *Ikzf3*. The two main domains of Aiolos include the N-terminal DNA-binding domain and the C-terminal dimerisation domain that allows interaction with other IKZF proteins such as Ikaros and Helios, as well as non-IKZF complexes[2]. Aiolos has been shown to play crucial roles in a wide variety of immune cells and responses by both directly promoting the transcription of specific genes, and indirectly silencing gene expression via chromatin remodelling[3]. Aiolos is expressed in most innate and adaptive cell types and has been shown to be important for the development of inflammatory ILCs in inflammatory bowel disease[4]. However, the role of Aiolos in the function of eosinophils and ILC2s has not been studied in detail.

Eosinophils primarily reside in the tissue following their exit from circulation in blood. It has been previously shown that Aiolos is highly expressed in eosinophils during eosinophil lineage commitment and maturation[5]. In this issue, Felton et al. expand on this observation and find that the absence of Aiolos results in reduced CCR3 protein expression in eosinophils in the small intestine (SI) and lung but not BM. Aiolos deficiency also impaired eosinophil accumulation in SI and the lungs during homeostasis with no effects observed in the BM, suggesting that Aiolos promotes the migration of eosinophils from the circulation to the mucosal tissues. Using two models of lung inflammation (ovalbumin-induced lung inflammation and inducible IL-13 transgenic mice), Felton et al. show that in the absence of Aiolos, eosinophils failed to accumulate in the airways, further highlighting that Aiolos controls the migration and residency of eosinophils in the lungs. Next, the authors use genome-wide expression analysis, coupled with histone modification chromatin immunoprecipitation sequencing and assay for transposase-accessible chromatin with sequencing (ATAC-seq) to identify the molecular targets of Aiolos in eosinophils. The authors find 371 differentially expressed genes between Aiolos-deficient and -sufficient eosinophils. These genes were enriched for chemotaxis, actin polymerisation and ERK/MAPK signalling pathways. Consistent with this, Aiolos deficiency impaired CCL11/CCR3-induced ERK1/2 activation, actin polymerisation and eosinophil migration in vitro and in vivo. ATAC-seq revealed that the transcriptional start site (TSS) regions of the differentially expressed genes were enriched for Aiolos binding motifs, including the proximal active enhancers of the *Ccr3* gene, suggesting a possible binding of Aiolos to these regions to regulate gene expression. In addition, the authors demonstrate that gene expression was strongly correlated with the activating histone H3 lysine 27 acetylation (H3K27ac) and H3K4me3 modifications within 1kB of the TSS of *Aiolos*, which are specific to eosinophils but not neutrophils. Predicted transcription factors that potentially bind to eosinophil-specific regions of *Aiolos* include PU.1, IKZF3 and GATA-1, all known to be important for eosinophil development and function. Importantly, the authors also find that human eosinophils also specifically express Aiolos and treatment of a human eosinophilic cell line with the Aiolos-targeting drug lenalidomide caused a reduction in Aiolos levels. Thus, Aiolos may be a novel therapeutic target to treat allergic diseases associated with tissue eosinophilia.

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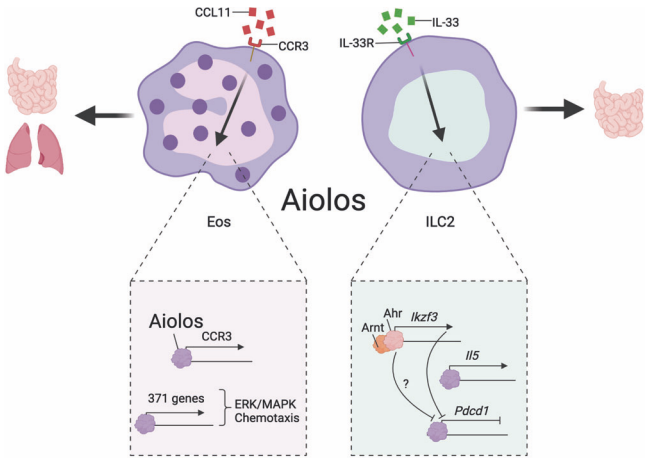

Fig. 1 Aiolos regulates tissue-resident eosinophils and ILC2s. In response to CCL11-CCR3 signals, Aiolos regulates CCR3 expression in eosinophils, as well as 371 genes that have been implicated in ERK/MAPK signalling pathways and chemotaxis. Aiolos controls the localisation of eosinophils in the small intestine and lungs. In contrast, IL-33 signals mediate the binding activity of the Arnt:Ahr transcription factor that promotes *Ikzf3* (encoding for Aiolos) expression in small intestine-resident ILC2s that in turns inhibits *Pdcd1* but is associated with *Il5* expression. ILC2 group 2 innate lymphoid cell. Created with BioRender.com.

In the second study, Qiu et al. identify a role for Aiolos in ILC2s through an unbiased single-cell RNA-sequencing (scRNA-Seq) approach. ILC2s are potent producers of type 2 cytokines such as IL-5 and IL-13 in the tissues and therefore are critical for protection against infection with parasitic worms[6]. However, dysregulated, ILC2 responses have been associated with allergy and asthma[6]. Qiu et al. used scRNA-seq to identify distinct transcriptomic profiles that are associated with ILC2s derived from distinct tissue sites including BM, large intestine (LI), SI, lung and pancreas. The authors find that there is significant heterogeneity between the tissue sites and observe that *Aiolos* is exclusively expressed in murine intestinal ILC2s and human colon ILC2s. SI and LI ILC2s have a shared core transcriptome that contains Aiolos, along with other transcription factors including the aryl hydrocarbon receptor (Ahr), the retinoic acid-inducible repressor Hic1 and Runx3. These factors have all previously been associated with tissue residency in the intestine[7–9]. Strikingly, adoptive transfer of ILC2s from different tissues into immunodeficient mice lacking *Rag2* and *Il2rg* revealed that intestinal ILC2s express high levels of Aiolos protein regardless of the tissue of origin of ILC2s, suggesting that Aiolos expression is a tissue-specific response to signals in the intestine. The authors go on to show that stimulation of Aiolos^{low} ILC2s with inflammatory cytokines including IL-33, TL1A and IL-1β results in upregulation of Aiolos expression. Thus, Aiolos is an intestinal tissue-specific marker that is induced by the intestinal microenvironment.

Using shRNA and CRISPR/Cas9 gene editing, the authors find a reduction in IL-5 but increased PD-1 expression in ILC2s upon *Ikzf3* knockdown, suggesting Aiolos directly promotes IL-5 expression while inhibiting PD-1 expression; an observation that is also consistent with IL-33-treated human ILC2s lacking Aiolos expression. The authors also observe that Aiolos-deficient ILC2s failed to recruit eosinophils to the intestine, which is likely due to impaired IL-5 expression. In addition to upregulated expression of *Ikzf3, Ahr* expression also appears to be highly specific to intestinal ILC2s. This prompted the authors to assess the impact of Ahr on Aiolos expression in ILC2s. Ligand activation of Ahr in vitro and in vivo results in increased expression of Aiolos with a concomitant decrease in PD-1 expression in ILC2s. Ahr-deficient ILC2s in long-term cultures (i.e., 10 days) express reduced IL-5 but increased PD-1 expression with impaired proliferative capacity, similar to Aiolos-deficient ILC2s. Mechanistically, Ahr controls the chromatin accessibility within *Ikzf3* locus (i.e., *Ikzf3*-+13 kB and *Ikzf3*-+19 kB), an observation that the authors find to be specific to ILC2s but not ILC1s and ILC3s. The authors further validate the binding of Ahr to the *Ikzf3* locus, which is associated with H3K27ac modifications. This study indicates that Ahr promotes Aiolos expression in ILC2s by promoting gene accessibility within *Ikzf3* locus and that targeting Aiolos in ILC2s could pave the way for novel interventions to alleviate ILC2-mediated inflammation.

The findings of Felton et al. and Qiu et al. both identify Aiolos as a critical factor for type 2 innate cell residency and function. Although it is clear that ILC2s and eosinophils have distinct developmental pathways, they tend to localise within similar tissues and can work synergistically to regulate the type 2 immune response. It is tempting to speculate that the same intestinal tissue signals may likely regulate the localisation and function of both eosinophils and ILC2s. While Qiu et al. find that pro-inflammatory cytokines such as IL-33 induce Aiolos expression in ILC2s, it would be interesting to determine whether or not IL-33 is also a potent inducer of Aiolos expression in eosinophils. Furthermore, as Ahr has been shown to regulate eosinophil function[10], the role of Ahr in Aiolos expression in eosinophils may also provide a common regulatory pathway for innate immune cell localisation in tissues. While the upstream signals of Aiolos could be universal across different cells that localise within the same tissue, the gene targets of Aiolos are likely to be distinct. In eosinophils, Aiolos regulates CCR3 expression, whereas Aiolos promotes IL-5 but represses PD-1 expression in ILC2s. Although Aiolos regulates expression of distinct genes in eosinophils and ILC2s, its mechanism of action is proposed to be similar, acting through the remodelling of chromatin. Cell type-specific factors that interact with Aiolos will likely modulate the downstream effects of Aiolos on gene expression and cellular function.

Taken together, the work by Felton et al. and Qiu et al. has shed light on the importance of Aiolos in tissue-specific eosinophils and ILC2s. A better understanding of both cell-intrinsic and -extrinsic factors that participate in the regulation of type 2 innate cells by Aiolos may provide new opportunities for better interventions to tackle diseases associated with dysregulated type 2 immune responses including asthma and allergy.

REFERENCES

1. Masopust, D. & Soerens, A. G. Tissue-resident T cells and other resident leukocytes. *Annu. Rev. Immunol.* **37**, 521–546 (2019).
2. John, L. B. & Ward, A. C. The Ikaros gene family: transcriptional regulators of hematopoiesis and immunity. *Mol. Immunol.* **48**, 1272–1278 (2011).
3. Heizmann, B., Kastner, P. & Chan, S. The Ikaros family in lymphocyte development. *Curr. Opin. Immunol.* **51**, 14–23 (2018).
4. Mazzurana, L. et al. Suppression of Aiolos and Ikaros expression by lenalidomide reduces human ILC3−ILC1/NK cell transdifferentiation. *Eur. J. Immunol.* **49**, 1344–1355 (2019).
5. Bouffi, C. et al. Transcription factor repertoire of homeostatic eosinophilopoiesis. *J. Immunol.* **195**, 2683–2695 (2015).
6. Lloyd, C. M. & Snelgrove, R. J. Type 2 immunity: expanding our view. *Sci. Immunol.* **3**, eaat1604 (2018).
7. Ye, J. et al. The aryl hydrocarbon receptor preferentially marks and promotes gut regulatory T cells. *Cell Rep.* **21**, 2277–2290 (2017).
8. Burrows, K. et al. HIC1 links retinoic acid signalling to group 3 innate lymphoid cell-dependent regulation of intestinal immunity and homeostasis. *PLoS Pathog.* **14**, e1006869 (2018).
9. Milner, J. J. et al. Runx3 programs CD8+ T cell residency in non-lymphoid tissues and tumours. *Nature* **552**, 253–257 (2017).
10. Tajima, H., Tajiki-Nishino, R., Watanabe, Y., Kurata, K. & Fukuyama, T. Activation of aryl hydrocarbon receptor by benzo[a]pyrene increases interleukin 33 expression and eosinophil infiltration in a mouse model of allergic airway inflammation. *J. Appl. Toxicol.* **40**, 1545–1553 (2020).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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