Biomarkers in severe asthma

What can biomarkers tell us about the pathological mechanisms that drive severe asthma and how might this manifest in patients?

Also available as a video featuring Professor Del Dorscheid discussing the key clinical biomarkers in severe asthma and what they can tell us about the inflammatory pathways underpinning disease.

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UNDERSTANDING THE CENTRAL ROLE OF THE EPITHELIUM IN SEVERE ASTHMA AND BEYOND

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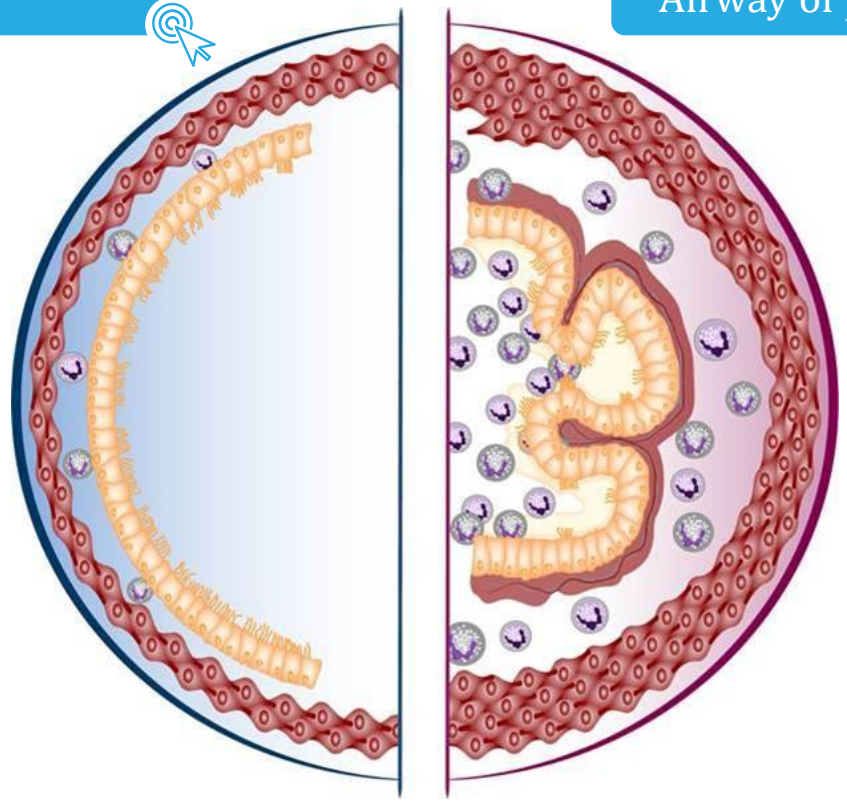
UNDERSTANDING THE CENTRAL ROLE OF THE EPITHELIUM IN SEVERE ASTHMA AND BEYOND

Healthy airway[1–4]

Airway of patient with severe asthma[2,3,5–11]



Abbreviations: IL, interleukin; TSLP, thymic stromal lymphopoietin.

References: 1. Holgate ST. Immunol Rev. 2011;242(1):205–219; 2. Bartemes KR and Kita H. Clin Immunol. 2012;143(3):222–235; 3. Roan F, et al. J Clin Invest. 2019;129(4):1441–1451; 4. Erle DJ and Sheppard D. J Cell Biol. 2014;205(5):621–631; 5. Wark PAB and Gibson PG. Thorax. 2006;61(10):909–915; 6. Likura M, et al. PLoS One. 2015;10(4):e0123584; 7. Baxi SN and Phipatanakul W. Adolesc Med State Art Rev. 2010;21(1):57–71; 8. Lambrecht BN and Hammad H. Nat Immunol. 2015;16(1): 45–56; 9. Heijink IH,et al. Allergy. 2020 Aug;75(8):1902–1917; 10. McBrien CN and Menzies-Gow A. Front Med (Lausanne). 2017;4:93; 11. Varricchi G, et al. Front Immunol. 2018;9:1595; 12. Gauvreau GM, et al. Expert Opin Ther Targets. 2020;24(8):777–792.

The role of the epithelium and epithelial cytokines in asthma

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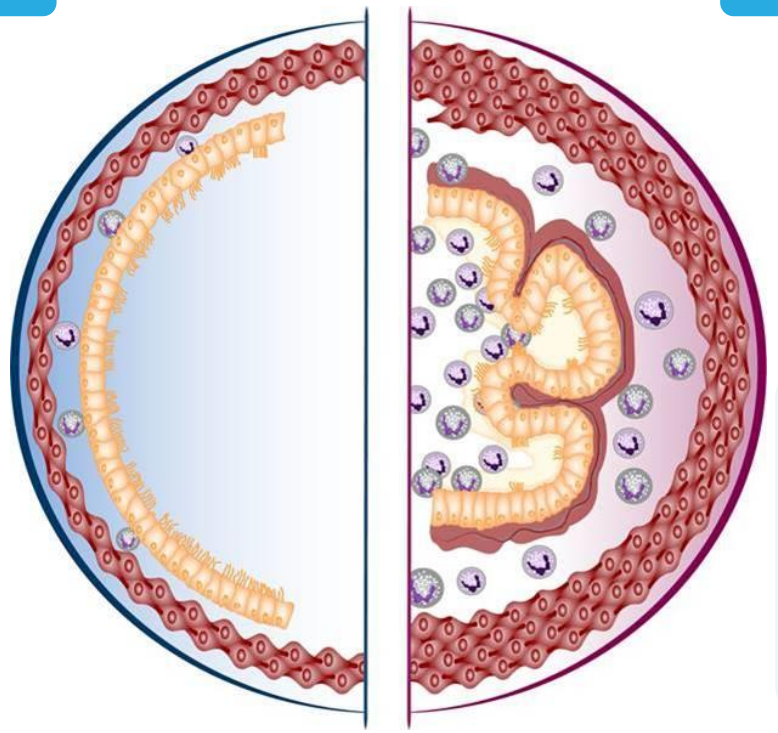
Healthy airway[1–4]

A highly regulated organ with a ciliated structure consisting of closely bound cells, which provides a physical barrier to environmental stimuli from the outside world[1]

- Acts as an **immune sensor** to the external environment through the controlled recruitment and activation of immune cells[2,3]
- Consists of several cell types including:[4]
  - **Goblet cells**, which produce and secrete mucus into the lumen
  - **Ciliated cells**, which propel mucus up the mucociliary ladder



Airway of patient with severe asthma[2,3,5–11]

- The airway epithelium may encounter several **inhaled triggers**, including pathogens (e.g. respiratory viruses or bacteria),[5,6] aeroallergens (e.g., pollen, house dust mites, animal dander and mould),[7–9] and irritants (e.g., cigarette smoke or air pollution, such as diesel particles)[8]
- In response to inhaled triggers, **the epithelium is altered** in a variety of ways. In asthma, the altered epithelium induces a **dysregulated response** and release of epithelial-derived cytokines (**IL-33**, **IL-25** and **TSLP**) that instigate a range of downstream inflammatory processes[2]
- These cytokines can create **a positive inflammatory feedback loop** and have been implicated in all three phenotypes of asthma[2,3,10–12]

Abbreviations: IL, interleukin; TSLP, thymic stromal lymphopoietin.

References: 1. Holgate ST. Immunol Rev. 2011;242(1):205–219; 2. Bartemes KR and Kita H. Clin Immunol. 2012;143(3):222–235; 3. Roan F, et al. J Clin Invest. 2019;129(4):1441–1451; 4. Erle DJ and Sheppard D. J Cell Biol. 2014;205(5):621–631; 5. Wark PAB and Gibson PG. Thorax. 2006;61(10):909–915; 6. Likura M, et al. PLoS One. 2015;10(4):e0123584; 7. Baxi SN and Phipatanakul W. Adolesc Med State Art Rev. 2010;21(1):57–71; 8. Lambrecht BN and Hammad H. Nat Immunol. 2015;16(1): 45–56; 9. Heijink IH,et al. Allergy. 2020 Aug;75(8):1902–1917; 10. McBrien CN and Menzies-Gow A. Front Med (Lausanne). 2017;4:93; 11. Varricchi G, et al. Front Immunol. 2018;9:1595; 12. Gauvreau GM, et al. Expert Opin Ther Targets. 2020;24(8):777–792.

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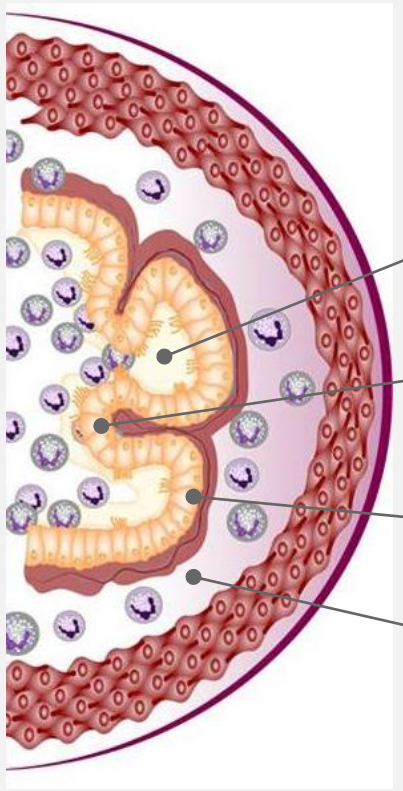

In response to inhaled triggers, the epithelium may be altered in a variety of ways:

Goblet cell hyperplasia and increased mucus production, resulting in mucus plugs[1,2]

Decreased epithelial tight-junction number and integrity, resulting in tissue damage as external insults penetrate the airway wall[2,3]

Increased basal membrane thickness via increased matrix deposition, resulting in airway narrowing[1,2,4]

Sub-epithelial inflammation and fibrosis can lead to fixed airway obstruction[3,4]

References: 1. Bartemes KR and Kita H. Clin Immunol 2012;143:222–235; 2. Holgate ST. Immunol Rev 2011;242(1):205–219; 3. Heijink IH, et al. Clin Exp Allergy 2014;44:620–630; 4.Cohen L, et al. Am J Respir Crit Care Med 2007;176:138–145.

The role of the epithelium and epithelial cytokines in asthma

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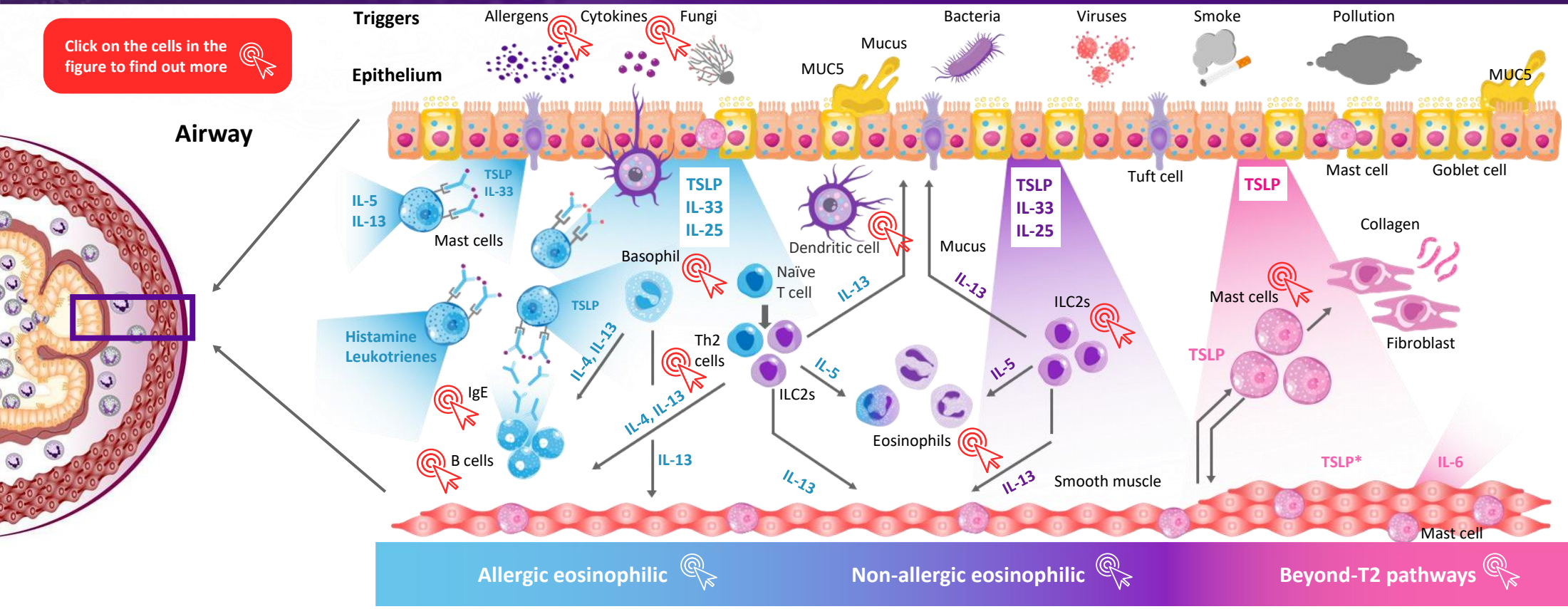
Click on the cells in the figure to find out more

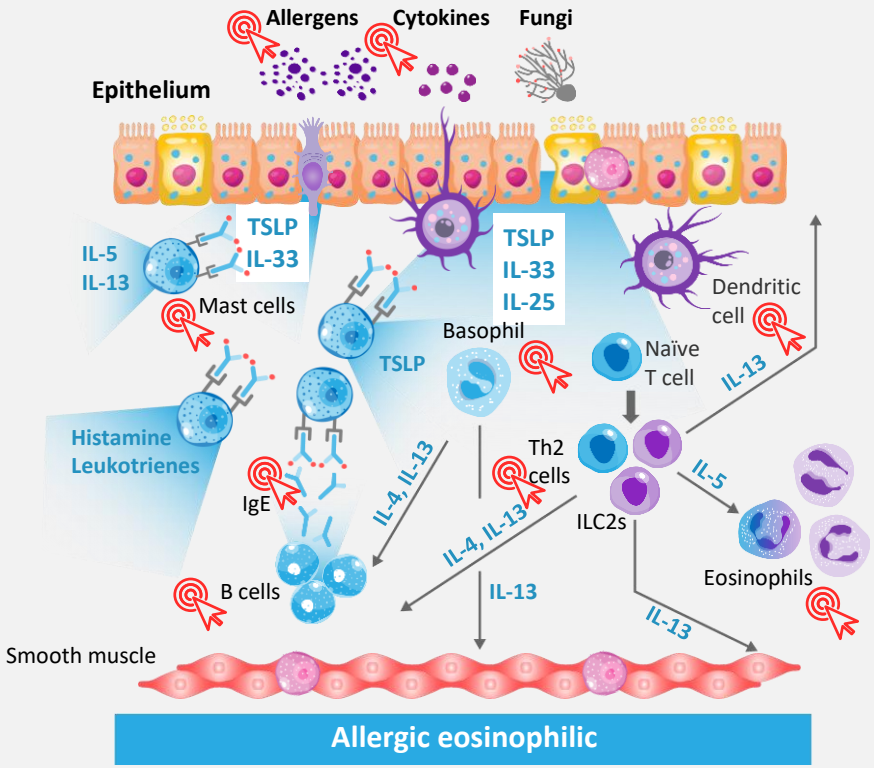


Airway remodelling describes structural changes related to basement membrane thickening, increased vascular density, airway smooth muscle thickening and subepithelial fibrosis[1]

Mechanisms underlying non-eosinophilic inflammation in asthma and the relevance of TSLP in its potential effects on macrophages both require further elucidation. The information presented in this image has been simplified for illustration purposes only and does not imply clinical benefit or relevance. Figure adapted from Gauvreau GM, et al. Expert Opin Ther Targets. 2020;24:777–792, which was based on Brusselle G, Bracke K. Ann Am Thorac Soc. 2014;11:S322–S328, Brusselle G, et al. Nat Med. 2013;19:977–979; Lambrecht BN, Hammad H. Nat Immunol. 2015;16:45–56; and Brightling CE, et al. N Engl J Med. 2021;385(18):1669–1679. Additional figure adaptations from Davis JD, et al. Mucosal Immunol. 2021;14:978–990. *Blockade of TSLP has suggested inhibition of pathways beyond type 2 inflammation. More recent evidence also suggests that Th1/17-driven neutrophilic pathways may not be key drivers of this type of inflammation.17–19 Abbreviations: IgE, immunoglobulin E; IL, interleukin; ILC2, type 2 innate lymphoid cell; T2, type 2; Th, T helper; TSLP, thymic stromal lympho poietin.

References: 1. Gauvreau GM, et al. Expert Opin Ther Targets. 2020;24:777–792; 2. Porsbjerg CM, et al. Eur Respir J. 2020;56:2000260; 3. Roan F, et al. J Clin Invest. 2019;129(4):1441–1451; 4. Varricchi G, et al. Front Immunol. 2018;9:1595; 5. Altman MC, et al. J Clin Invest. 2019;129:4979–4991; 6. Allakhverdi Z, et al. J Exp Med. 2007;204:253–258; 7. Kato A, et al. J Immunol. 2007;179:1080–1087; 8. Kouzaki H, et al. J Immunol. 2009;183(2)1427–1434; 9. Cohn L. J Clin Invest. 2006;116(2):306–308; 10. Brusselle GG and Koppelman GH. N Engl J Med. 2022;386(2):157–171; 11. Janeway ICA et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 12. Davis JD and Wypych TP. Mucosal Immunology. 2021;14:978–990; 13. Brightling CE, et al. N Engl J Med. 2002; 346:1699–1705; 14. Begueret H, et al. Thorax. 2007;62:8–15; 15. Krystel-Whittemore M, et al. Front Immunol. 2016;6:620; 16. Gao H et al. J Immunol Res. 2017;3743048; 17. Diver S, et al. Lancet Respir Med. 2021;9(11):1299–1312; 18. Sverrild A, et al. Eur Respir J. 2022;59:2101296; 19. Brightling CE, et al. N Eng J Med. 2021;385:1669–1679.

Allergic eosinophilic asthma (type 2 response)



- Triggers of allergic eosinophilic asthma (e.g. allergens, cytokines and fungi) interact with the airway epithelium, and this may cause injury that initiates the release of epithelial cytokines, such as **IL-33, IL-25 and TSLP**[1,2]
- These cytokines activate a pathway resulting in smooth muscle contraction, which can contribute to airway hyperresponsiveness, and eosinophilic inflammation[1–7]
- Allergic eosinophilic asthma is characterised by **biomarkers** including high blood eosinophils, high FeNO levels and high serum IgE[1,8,9]

Figure adapted from Porsbjerg CM, et al. Eur Respir J. 2020;56:2000260 and Gauvreau GM, et al. Expert Opin Ther Targets. 2020 ;24:777–92, which was based on Brusselle G and Bracke K. Ann Am Thorac Soc. 2014;11:S322–S328, Brusselle G, et al. Nat Med. 2013;19:977–79 and Lambrecht BN and Hammad H. Nat Immunol. 2015;16:45–56.
Abbreviations: IgE, immunoglobulin E; IL, interleukin; ILC2, type 2 innate lymphoid cell; T2, type 2; Th, T helper; TSLP, thymic stromal lymphopoietin.
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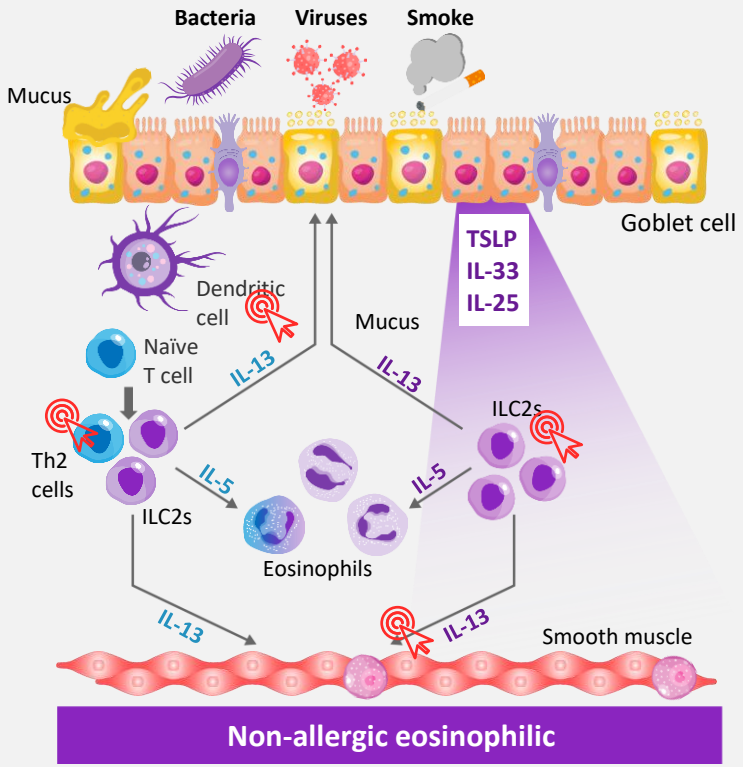
Non-allergic eosinophilic asthma (type 2 response)



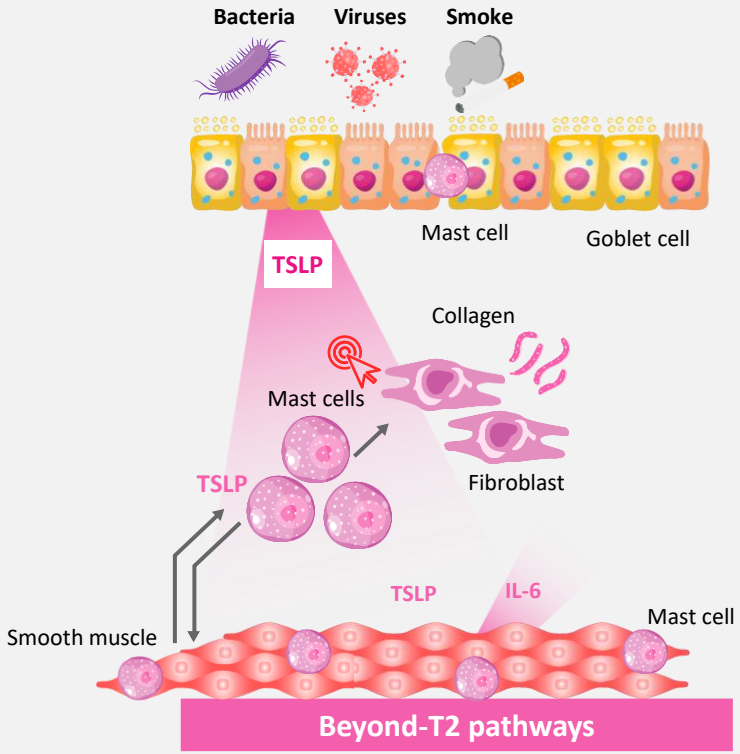
- Triggers of non-allergic eosinophilic asthma (e.g. bacteria, viruses and smoke),[1-3] interact with the airway epithelium, and this may cause injury that initiates the release of epithelial cytokines, such as **IL-33, IL-25 and TSLP**[3]
- These cytokines activate a pathway resulting in inflammation, smooth muscle contraction, airway hyperresponsiveness, and mucus overproduction leading to the formation of mucus plugs[3–7]
- Non-allergic eosinophilic asthma is characterised by **biomarkers** including high blood eosinophils and high FeNO levels[1–3]

Figure adapted from Porsbjerg CM, et al. Eur Respir J. 2020;56:2000260 and Gauvreau GM, et al. Expert Opin Ther Targets. 2020 ;24:777–92, which was based on Brusselle G and Bracke K. Ann Am Thorac Soc. 2014;11:S322–S328, Brusselle G, et al. Nat Med. 2013;19:977–79 and Lambrecht BN and Hammad H. Nat Immunol. 2015;16:45–56.

Abbreviations: FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E; IL, interleukin; ILC2, type 2 innate lymphoid cell; T2, type 2; Th, T helper; TSLP, thymic stromal lymphopoietin.

References: 1. Gauvreau GM, et al. Expert Opin Ther Targets. 2020;24(8):777–792; 2. Oppenheimer J, et al. Ann Allergy Asthma Immunol. 2022;129:169–180; 3. Walford HH, et al. J Asthma Allergy. 2014;7:53–65; 4. Jia Y, et al. Am J Respir Cell Mol Biol. 2016;55(5):675–683; 5. Marone G, et al. Front Pharmacol. 2019;10:1387; 6. Escamilla-Gil JM, et al. Biomed Res Int. 2022. doi: 10.1155/2022/5753524; 7. Dunican EM, et al. J Clin Invest. 2018;128(3):997–1009.

Beyond-T2 asthma (beyond-T2 response)



- Bacteria, viruses, smoke, and non-allergic particles from the urban environment interact with the airway epithelium, and this may cause injury that initiates the release of epithelial cytokines, such as **TSLP**[1–4]
- These cytokines may result in the activation of pathways beyond the T2 response, and result in mechanistic effects in the absence of T2 inflammation that characterise this asthma subtype. These include airway remodelling, airway hyperresponsiveness, and mucus production[3–10]

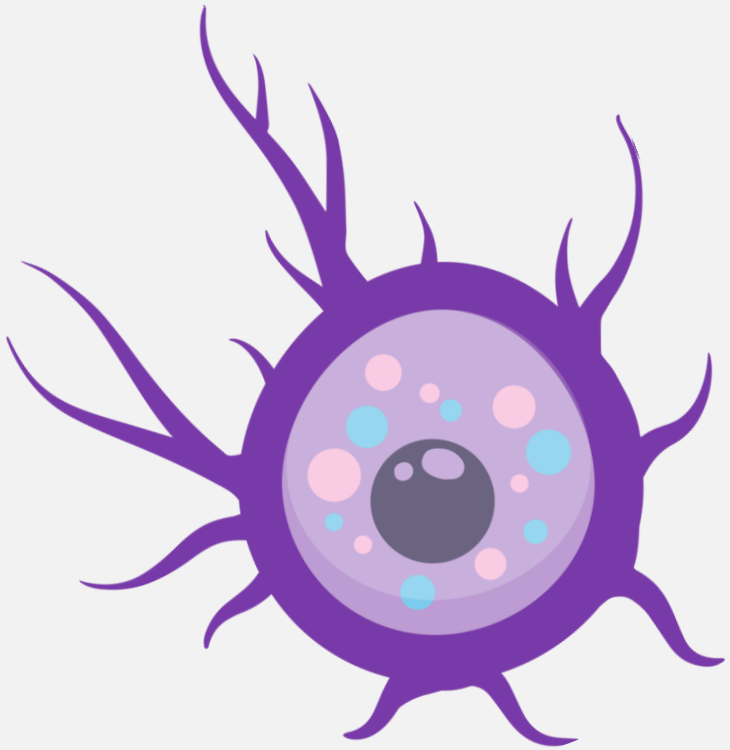
Figure adapted from Porsbjerg CM, et al. Eur Respir J. 2020;56:2000260 and Gauvreau GM, et al. Expert Opin Ther Targets. 2020;24:777–92, which was based on Brusselle G and Bracke K. Ann Am Thorac Soc. 2014;11:S322–S328, Brusselle G, et al. Nat Med. 2013;19:977–79 and Lambrecht BN and Hammad H. Nat Immunol. 2015;16:45–56.

*Blockade of TSLP has suggested inhibition of pathways beyond type 2 inflammation. More recent evidence also suggests that Th 1/17-driven neutrophilic pathways may not be key drivers of this type of inflammation.[8–10]

Abbreviations: IgE, immunoglobulin E; IL, interleukin; ILC2, type 2 innate lymphoid cell; T2, type 2; Th, T helper; TSLP, thymic stromal lymphopoietin.

References: 1. Gauvreau GM, et al. Expert Opin Ther Targets. 2020;24:777–792; 2. Hudey SN, et al. Curr Opin Immunol. 2020;66:123–128; 3. Renauld CJ. J Clin Pathol. 2001;54:577–589; 4. West EE, et al. Drug Discov Today Dis Mech. 2012;9:3–4; 5. Shan L, et al. J Immunol. 2010;184:7134–7143; 6. Berair R, et al. BMC Medicine. 2013; 11:145; 7. Page S, et al. Am J Physiol Lung Cell Mol Physiol. 2001;281: L1313–L1323; 8. Brightling CE, et al. N Eng J Med. 2021;385: 1669–79; 9. Diver S, et al. Lancet Respir Med. 2021;9(11):1299–1312; 10. Sverrild A, et al. Eur Respir J. 2022;59:2101296.

Dendritic cell

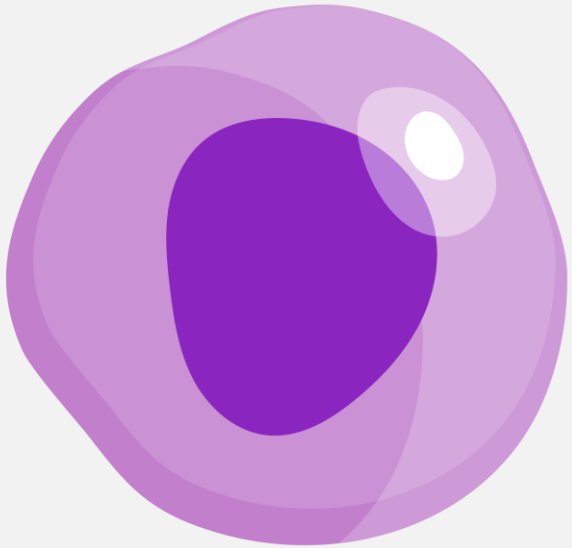


- Dendritic cells are innate immune cells which engulf and process antigen peptides for presentation to T cells[1,2]
- Dendritic cells are the most potent T-cell stimulators and attract and activate naïve T cells[3]
- They can express several molecules that attract basophils, eosinophils and T cells[1]
- Dendritic cells can be divided into plasmacytoid (pDC) and monocyte-derived conventional (cDC1 and cDC2) subsets.[1,4] In particular, conventional DCs promote Th2 responses by secreting proinflammatory cytokines and upregulating the expression of costimulatory molecules after antigen stimulation[1]

Abbreviations: Th, T helper.

References: 1. Kim HY, et al. Nat Immunol. 2010;11(7)577–584; 2. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 3. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27118/ [Accessed September 2021]; 4. Collin M, et al. Immunology. 2018;154(1):3–20

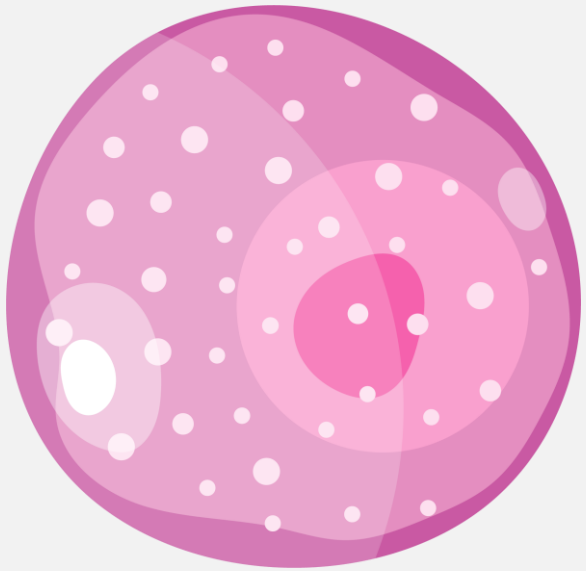
ILC2 cell



- Type 2 innate lymphoid cells (ILC2) play an important role in response to certain aeroallergen threats in the absence of adaptive immune cells such as T cells[1,2]
- ILC2 cells can be activated by IL-25, IL-33 and TSLP[1,2]
- They produce IL-5 which can lead to the recruitment and activation of eosinophils and IL-13 which can cause secretion of mucus from goblet cells[1,2]

Abbreviations: IL, interleukin; ILC2, type 2 innate lymphoid cells; TSLP, thymic stromal lymphopoietin.
References: 1. Licona-Limón P, et al. Nat Immunol. 2013;14(6):536-542; 2. Salter BM, et al. J Leukoc Biol. 2019;106:889–901.

Mast cell



- Mast cells contain granules which store a variety of molecules (including histamine and leukotrienes)[1,2]
- When the antibody IgE binds to a surface receptor, granule release is triggered, resulting in a hypersensitivity reaction[1,2]
- Mast cells can also be activated by TSLP, IL-25 and IL-33[2,3]

Abbreviations: IgE, immunoglobulin E; IL, interleukin; TSLP, thymic stromal lymphopoietin.

References: 1. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 2. Stone KD, et al. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S73–S80; 3. Walford HH, et al. J Asthma Allergy. 2014;7:53–65.

Basophil

- Basophils contain granules that store a variety of molecules (including histamine and potent bronchoconstrictors)[1,2]
- These cells can be activated via IgE binding, causing the secretion of cytokines IL-4 and IL-13 that promote Th2 differentiation[1,2]
- TSLP is understood to directly influenced basophil activation as well[3]

Abbreviations: IgE, immunoglobulin E; IL, interleukin; Th2, T-helper 2 cell; TSLP, thymic stromal lymphopoietin.

References: 1. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 2. Stone KD, et al. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S73–S80; 3. Gauvreau GM, et al. Expert Opin Ther Targets 2020;24(8):777–792.

Eosinophil

- Eosinophils contain granules that store a variety of proinflammatory molecules (including cationic proteins)[1]
- Their development and ingress is promoted by cytokines (IL-5) and they release various lipid mediators and cytokines[1]
- Eosinophils are **biomarkers** of asthma[2]

Abbreviations: IL, interleukin.
References: 1. Stone KD, et al. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S73–S80; 2. Wan XC, et al. Immunol Allergy Clin North Am 2016:36(3);547–557.

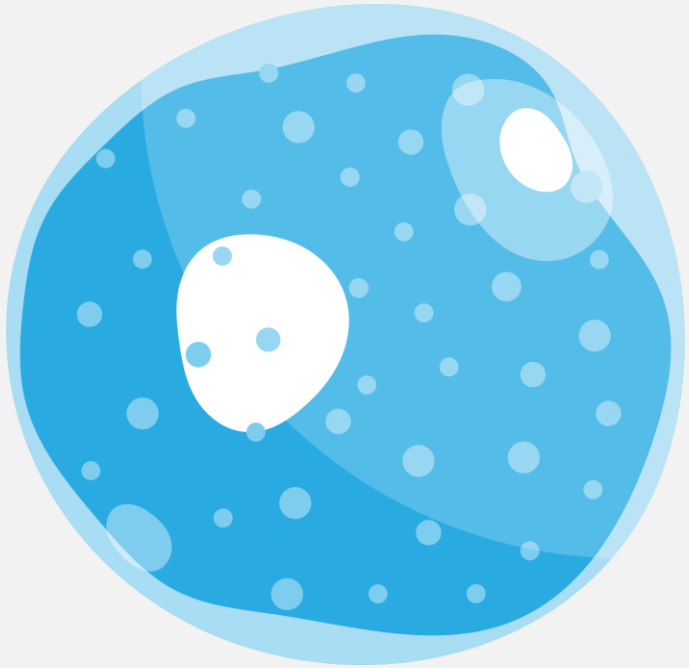
T cell

- T cells are adaptive immune cells that are activated when antigens are presented to them by other cells (e.g. dendritic cells and macrophages).[1,2,3] Several subsets of T cells exist:
  - **Th2 cells**: develop in the presence of IL-4[4]. They produce IL-4 and IL-5, thereby stimulating B cells to produce IgE and promote the recruitment of mast cells and eosinophils[2,5,6]
  - **Th1 cells**: develop in the presence of IL-12 and produce proinflammatory cytokines (IFN-γ) which can promote mast cell activation in animal models[7]
  - **Th17 cells**: develop in the presence of cytokines IL-6 and TGF-β and produce proinflammatory cytokines (particularly IL-17) which can attract the innate immune neutrophils and plays a role in promoting airway hyperresponsiveness[5,7]

Abbreviations: IFN-γ, interferon gamma; IgE, immunoglobulin E; IL, interleukin; TGF-β, transforming growth factor beta.

References: 1. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 2. Janeway CA, et al. Annu. Rev. Immunol. 2002. 20:197–216; 3. Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. Helper T Cells and Lymphocyte Activation and T Cells and MHC Proteins. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/ [Accessed September 2023]; 4. Yoshimoto T. Front Immunol. 2018;9:716; 5. Gauvreau GM, et al. Expert Opin Ther Targets 2020;24(8):777–792; 6. Kim HY, et al. Nat Immunol. 2010;11(7)577–584; 7. Luo W, et al. Front. Immunol. 2022;13:974066.

B cell



- B cells are adaptive immune cells which are activated following an encounter with an antigen binding to a surface receptor antibody. This results in them producing antibody molecules of the same antigen specificity as the receptor [1–3]
- Following interaction with a T helper cell, the B cells can switch from producing one form of antibody (e.g. IgG) to another form of antibody (IgE)[1–3]
- Activated B cells can turn into plasma cells or memory cells[1–3]
  - **Plasma cells**: These B cells secrete antigen-specific antibodies into the environment
  - **Memory B cells**: These B cells are responsible for immunological memory; they can respond rapidly to produce antigen-specific antibody when a threat re-emerges

Abbreviations: IgE, immunoglobulin E; IgG, immunoglobulin G.

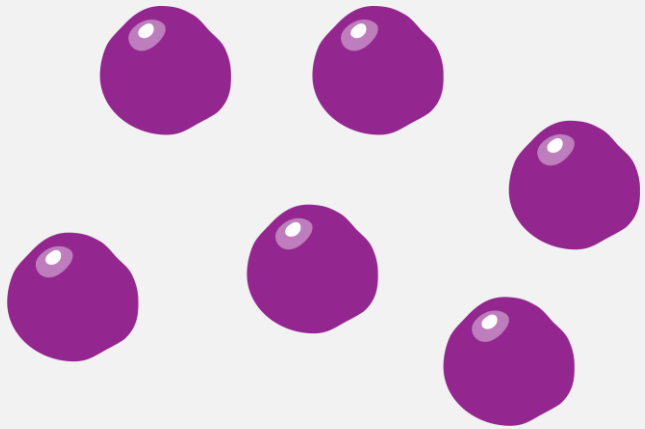
References: 1. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 2. Scott-Taylor TH. Immun Inflamm Dis. 2017;6(1):13–33; 3. Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. B Cells and Antibodies. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/ [Accessed August 2023].

Allergen

- A molecule (generally one that can bind to an antibody) that elicits an allergic reaction or hypersensitivity[1]
- For patients with asthma, allergens can include dust mite and cockroach faeces, animal dander (e.g. hair, skin or feathers), fungus (e.g. *Alternaria*), and pollen (e.g. from trees, grasses, and weeds)[2,3]
- A single source can produce multiple allergens, and therefore measuring sensitivity or finding a specific antibody in asthma diagnosis and management is difficult[2]

References: 1. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 2. Baxi SN and Phipatanakul W. Adolesc Med State Art Rev. 2010;21:57–71; 3. Lambrecht BN and Hammad H. Nat Immunol. 2015;16(1):45–56.

Cytokine



- Proteins produced by cells that can affect the behaviour of other cells when they bind to specific receptors (e.g. IL-4, IL-5, IL-13, IL-25)[1]
- Cytokines play a variety of roles in the immune response, including cell activation and migration as well as cellular differentiation (e.g. IL-25, along with IL-4, can drive Th2 cell differentiation)[1,2]

Abbreviations: IL, interleukin; Th, T helper.

References: 1. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 2. Roan F, et al. J Clin Invest. 2019;129(4):1441–1451.

Antibody

- A protein (such as IgE or IgG) produced by B cells that binds specifically to a particular substance (an antigen/allergen) and can neutralise it (in the case of an infecting microbe) or prepares it for ingestion and destruction by other cells[1]
- IgE is generally produced in response to an allergic reaction and can bind to receptors located on mast cells and basophils, triggering the release of molecules such as histamine[2,3]
- Antibodies are **biomarkers** of asthma[4]

Abbreviations: IgE, immunoglobulin E; IgG, immunoglobulin G.

References: 1. Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/ [Accessed August 2023]; 2. Scott-Taylor TH. Immun inflamm dis. 2017;6(1):13–33; 3. Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. B Cells and Antibodies. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/ [Accessed August 2023]; 4. Kim H, et al. Allergy Asthma Clin Immunol 2017;13:48: doi: 10.1186/s13223-017-0219-4.

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- Asthma Biomarkers
- Fractional Exhaled Nitric Oxide (FeNO)
- Pulmonary Function Test (PFT)
- Methacholine Challenge Test
- Beta-Agonist Reversibility
- CT Scan
- Sputum Sample
- Sputum Induction
- Bronchoscopy
- Questionnaires

Abbreviations: CT, computerised tomography; FeNO, fractional exhaled nitric oxide; PFT, pulmonary function test.

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Asthma Biomarkers

Test for changes at the cellular level

Validated biomarkers

- Eosinophils
- *Aspergillus* Precipitins
- IgE
- CRP
- FeNO
- ANCA and ANA

Experimental biomarkers; clinical utility not yet demonstrated

- Periostin
- Airway Mucin Analysis

Abbreviations: ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody; CRP, C-reactive protein; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E.

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Asthma Biomarkers

Test for changes at the cellular level

[Explore the role of eosinophils in the inflammatory cascade]

Eosinophils

Validated asthma biomarker

- Identifies eosinophilic asthma[1]
- Eosinophils may be measured peripherally (in the blood) or within sputum[1,2]
  - The latter is considered a more accurate representation of asthma, however, blood eosinophils are easier to obtain and may be a potential surrogate biomarker[1–3]

References: 1. Wan XC, et al. Immunol Allergy Clin North Am 2016:36(3);547–557; 2. Pizzichini E, et al. J Allergy Clin Immunol 1997;99(4):539–544; 3. Lombardi C, et al. Curr Res Immunol. 2022;3:42–53.

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Asthma Biomarkers

Test for changes at the cellular level

[Explore the role of IgE in the inflammatory cascade]

IgE

Validated biomarker

- Is the predominant biomarker for allergic asthma[1]
- Measured via blood sample[1]
  - Serum IgE levels have been shown to correlate closely with the severity of asthma across all ages[1]
  - Serum IgE levels are also associated with airway hyper-responsiveness, even in patients without a history of asthma symptoms or atopy[1]

Abbreviations: IgE, immunoglobulin E.

References: 1. Kim H, et al. Allergy Asthma Clin Immunol 2017;13:48: doi: 10.1186/s13223-017-0219-4.

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Asthma Biomarkers

Test for changes at the cellular level

[Explore the role of eosinophils in the inflammatory cascade]

Fractional Exhaled Nitric Oxide (FeNO)

Validated biomarker

- Identifies type 2 inflammation asthma; increased FeNO may be associated with a greater risk of asthma exacerbations[1,2]
- A FeNO test may be helpful for:[1,2]
  - Detecting eosinophilic airway inflammation
  - Predicting risk of exacerbations and lung function decline
  - Determining steroid responsiveness
  - Assessing adherence to inhaled corticosteroid therapy
- Measured via exhaled air[2] and can be performed in the clinic at each visit[3]

Abbreviations: FeNO, fractional exhaled nitric oxide.

References: 1. Kim H, et al. Allergy Asthma Clin Immunol 2017;13:48: doi: 10.1186/s13223-017-0219-4; 2. Wan XC, et al. Immunol Allergy Clin North Am 2016:36(3);547–557; 3. Bernholm KF, et al. ERJ Open Res. 2018;4(4): 00147-2017.

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Asthma Biomarkers

Test for changes at the cellular level

[Explore the role of IgE in the inflammatory cascade]

Aspergillus precipitins

Validated biomarker

- Aspergillus precipitins are a type of IgG, resulting from exposure to the fungus *aspergillus*[1,2]
- Presence or growth of *aspergillus* (as well as other fungi that can cross react) in the lungs causes the IgG response. Then subsequent exposure generates inflammation, as seen in allergic bronchopulmonary aspergillosis asthma (ABPA)[2,4]
- There is no definitive test to diagnose ABPA or consensus on the combination of diagnostic criteria[2,3]

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis asthma; Ig, immunoglobulin.

References: 1. Richardson MD and Page ID. Med Mycol. 2017;55(1):48-55; 2. Jack J, Bajaj T. Allergic bronchopulmonary aspergillosis. StatPearls [Internet]. StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK542329. [Accessed August 2023]; 3. Moral L, et al. Allergol Immunopathol (Madr). 2019;47(2):107–121; 4. Agarwal R, et al. Indian J Med Res. 2020;151(6):529–49.

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Asthma Biomarkers

Test for changes at the cellular level

[Explore the role of IL-6 in the inflammatory cascade]

CRP

Validated biomarker

- Helps identify low eosinophil count and non-allergic asthma[1]
- hs-CRP helps detect the severity of asthma[1]
- Effects could be characterised by structural changes to the lung including airway inflammation and resistance to steroid treatment[1,2]
- Measured via blood sample; specifically, hs-CRP may be measured as it is a surrogate marker for cytokine IL-6[1,3]

Abbreviations: CRP, c-reactive protein; hs-CRP: high-sensitivity CRP; IL-6, interleukin 6.

References: 1. Ong LT, et al. EMJ Allergy Immunol. 2021;6(1):53–60; 2. Monadi M, et al. Caspian J Intern Med. 2016 Winter;7(1):37–42; 3. Denton CP and Ong VH. J Scleroderma Relat Disord. 2017;2(2_suppl):S13–S19.

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Asthma Biomarkers

Test for changes at the cellular level

ANCA and ANA

Validated biomarker

- Helps identify the presence of antineutrophil cytoplasmic antibodies (ANCAs) and positive antinuclear antibodies (ANAs), which may be present in patients with asthma[1,2,3]
- The identification of these auto-antibodies may help identify complex and severe asthma phenotypes and endotypes[4]
- Measured via blood sample[1]

Abbreviations: ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody.

References: 1. MedLine Plus. Antineutrophil cytoplasmic antibodies (ANCA) test 2021. Available from: medlineplus.gov/lab-tests/antineutrophil-cytoplasmic-antibodies-anca-test. [Accessed August 2023].; 2. MedLine Plus. Antinuclear antibodies (ANA) test 2022. Available from: medlineplus.gov/lab-tests/ana-antinuclear-antibody-test. [Accessed August 2023].; 3. Grygiel-Gorniak B, et al. Reumatologia.2017;55(6):298–305; 4. Mukherjee M, Nair P. Allergy Asthma Immunol Res. 2018;10(5):428–447.

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Asthma Biomarkers

Test for changes at the cellular level

Periostin

Experimental biomarker; clinical utility not yet demonstrated

- May be a surrogate biomarker for type 2 inflammation and tissue remodelling in asthma, and could help in identifying endotypes such as hyperresponsiveness' to inhaled corticosteroids[1,2]
- Appears to be correlated to eosinophils and FeNO in severe asthma[1]
- May facilitate the identification of patients with uncontrolled asthma who may respond to certain biologic treatments[3]
- Measured via blood sample[1]

Abbreviations: FeNO, fractional exhaled nitric oxide.

References: 1. Izuhara K, et al. Allergy Asthma Immunol Res. 2016;8(6):491–498; 2. Izuhara K, et al. Allergy. 2019;74(11):2116–2128; 3. Matsumoto H. Respir Investig. 2020;58(3):144-54.

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Asthma Biomarkers

Test for changes at the cellular level

Airway Mucin Analysis

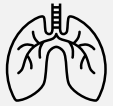
Experimental biomarker; clinical utility not yet demonstrated

- Mucins are glycoproteins produced by goblet cells in the epithelium and by sero-mucus glands in the submucosa[1]
- Accumulation analysis of mucins may be an indicator of various phenotypes of asthma (i.e., non-allergic), especially those with overproduction of mucus and the formation of mucus plugs[2,3]

References: 1. Santus P, et al. J Clin Med. 2019;8(11):1955; 2. Crespo-Lessmann A, et al. J Asthma Allergy. 2017;10:269–276; 3. Gauvreau GM, et al. Expert Opin Ther Targets 2020;24(8):777–792.

Clinical evaluations for asthma

Pulmonary Function Test (PFT)



Tests lung function

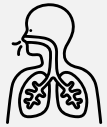
Spirometry may be used to assess pulmonary function, using the following tests:[1]

- Forced expiratory volume in one second (FEV_1)[1]
- Forced vital capacity (FVC) – the maximum amount of air that can be exhaled when blowing out as fast as possible[1]
- Residual volume (RV) – the volume of air remaining in the lungs after a maximal expiration[2]

Spirometry and the calculation of FEV_1/FVC allows the identification of obstructive or restrictive ventilatory defects; RV may be significantly increased in patients with obstructive lung diseases, where there is incomplete emptying of the lungs and air trapping[2]

Abbreviations: PFT, pulmonary function test; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; RV, residual volume.
References: 1. Moore VC. Breathe. 2012;8:232–240; 2. Ranu H, et al. Ulster Med J. 2011;80(2):84–90.

Methacholine Challenge Test



Tests airway function and ability to acutely spasm

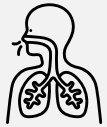
Methacholine challenge

- During a methacholine challenge test (or a bronchoprovocation test), the patient inhales doses of methacholine[1,2]
- Inhaled methacholine directly induces airway smooth muscle constriction, leading to a measurable reduction in lung expiratory volume[1,2]
- A breathing test is repeated after each dose of methacholine to measure the degree of narrowing or constriction of the airways. If 20% of the pre-test FEV_1 is lost during this test, further doses are not required, and the test is declared positive for the diagnosis of asthma[1–3]

Abbreviations: FEV_1, forced expiratory volume in one second.

References: 1. Sverrild A, et al. Respir Res. 2021;22:287; 2. Sverrild A, et al. J Allergy Clin Immunol. 2010;126:952–958; 3. Global Strategy for Asthma Management and Prevention. 2023. Available from: www.ginasthma.org . [Accessed August 2023].

Beta-agonist Reversibility



Tests reversibility of airway remodelling and the ability to spasm

- Bronchodilator reversibility measures the increase in expiratory airflow in response to an inhaled short-acting bronchodilator and is usually based on the change in FEV_1[1]
- The Global Initiative for Asthma (GINA) recommends spirometry to assess bronchodilator reversibility as the first-line in the investigation of asthma[1]
- For a diagnosis of asthma, the cut-off recommended by current international guidelines is a change in FEV1 ≥12% from baseline and ≥200 mL[2]

Abbreviations: FEV_1, forced expiratory volume in one second.
Reference: 1. Tan DJ, et al. ERJ Open Res. 2021;7:00042–2020; 2. Global Strategy for Asthma Management and Prevention. 2023. Available from: www.ginasthma.org [Accessed August 2023].

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CT Scan

Allows visualisation of chest or sinuses

- For patients with persistent asthma symptoms, a chest CT scan provides an assessment of the lung and airway structure, which should be evaluated in combination with results of lung function tests, and possibly bronchoscopy or sputum sample/induction results[1,2]

Abbreviations: CT, computerised tomography.

References: 1. Walker C, et al. Curr Opin Pulm Med. 2012;18(1):42–47; 2. Global Strategy for Asthma Management and Prevention. 2023. Available from: www.ginasthma.org [Accessed August 2023].

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Sputum Sample

Tests for changes at the cellular level

- Sputum is a substance produced in the airways as a reaction to irritation and inflammation and is also known as mucus or phlegm[1]
- Once collected, sometimes via **sputum induction**, it can be processed in a typical hospital laboratory setting[2]
- Sputum analysis is commonly used to detect tuberculosis, but in recent years has become a standard method to diagnose asthma and to monitor treatment as well; analysis of white blood cells found in the sputum of asthma patients has been found to be a highly reliable way to determine asthma type[1–3]
- Sputum may also be tested for **fungus**, and other microbiological and mycobacterial cultures to further assess for complex causes of uncontrolled asthma[1]

References: 1. Shen F, Sergi C. Sputum analysis. StatPearls [Internet]. StatPearls Publishing; January 2023. www.ncbi.nlm.nih.gov/books/N BK563195; 2. Keun Kim C, Hagan JB. Ann Allergy Asthma Immunol. 2004;93(2):112–22; 3. Davies AR. Curr Opin Pulm Med. 2013:19(1):60–65.

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Sputum Induction

Tests for changes at the cellular level

- Some asthma patients can produce sputum spontaneously, but in such cases the viability of the cells collected is poor, making analysis of cell type difficult[1]
- Therefore, sputum collected to identify the inflammatory process present in the airways is usually obtained through a procedure called sputum induction, whereby the patient inhales a nebulised saline solution (a mist) which triggers a reflex cough due to fluid secretions from the airways[1]
- The procedure is minimally invasive, and studies have shown it to be effective in the vast majority of patients, with a low risk of an adverse reaction[1,2]

References: 1. Davies AR, Hancox RJ. Curr Opin Pulm Med. 2013;19(1):60–65; 2. Pizzichini E, et al. Eur Respir J Suppl. 2002;37:9s-18s.

Bronchoscopy

Tests for changes at the cellular level

- A procedure to inspect the airways for possible explanations for persistent symptoms[1]
- Tests include cytology and identification of immune cells from the airways and from cultures of mucus samples and washings[2,3]
- Bronchoscopy, along with sputum induction, can also be used to check for fungi and bacteria, including mycobacteria in the lungs[2,3]

References: 1. Moore WC, et al. J Allergy Clin Immunol. 2011;28(2):328–336; 2. Ben Tkhayat R, et al. ERJ Open Res 2021;7:00332-2021; 3. Huang C, et al. Front Pediatr. 2022;10:822043.

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Questionnaires

Questionnaires can be used to document symptoms and quality of life or to identify changes in response to treatment(s)

Asthma Control Questionnaire[1]
Measures adequacy of control and changes to control

St George's Respiratory Questionnaire[2]
Measures impact on overall health, daily life, and perceived well-being

Asthma Control Test[3]
Patient self-administered tool for identifying those with poorly controlled asthma

Asthma Quality of Life Questionnaire[4]
A tool used to assess the physical, occupational, emotional, and social qualities of with asthma

Severe Asthma Questionnaire[5]
A tool used to measure patient quality of life, including evaluation of disease and treatment

References: 1. Asthma Control Questionnaire. Available from: https://www.thoracic.org/members/assemblies/assemblies/srn/questionaires/acq.php. [Accessed August 2023]; 2. St George's Respiratory Questionnaire. Available from: https://www.thoracic.org/members/assemblies/assemblies/srn/questionaires/sgrq.php. [Accessed August 2023]; 3. Asthma Control Test. Available from: https://www.thoracic.org/members/assemblies/assemblies/srn/questionaires/act.php. [Accessed August 2023]; 4. Asthma Quality of Life Questionnaire. Available from: https://www.thoracic.org/members/assemblies/assemblies/srn/questionaires/aqlq.php. [Accessed August 2023]; 5. Severe Asthma Questionnaire. Available from: http://saq.org.uk/Download/SAQ.pdf. [Accessed August 2023].

References [1/3]

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UNDERSTANDING THE CENTRAL ROLE OF THE EPITHELIUM IN SEVERE ASTHMA AND BEYOND

- Agarwal R, et al. Indian J Med Res. 2020;151(6):529–49.
- Alberts B, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. B Cells and Antibodies. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/.
- Allakhverdi Z, et al. J Exp Med. 2007;204:253–258.
- Altman MC, et al. J Clin Invest. 2019;129:4979–4991.
- Asthma Control Questionnaire. Available from: https://www.thoracic.org/members/assemblies/assemblies/srn/questionaires/acq.php.
- Asthma Control Test. Available from: https://www.thoracic.org/members/assemblies/assemblies/srn/questionaires/act.php.
- Asthma Quality of Life Questionnaire. Available from: https://www.thoracic.org/members/assemblies/assemblies/srn/questionaires/aqlq.php.
- Bartemes KR and Kita H. Clin Immunol. 2012;143(3):222–235.
- Baxi SN and Phipatanakul W. Adolesc Med State Art Rev. 2010;21(1):57–71.
- Begueret H, et al. Thorax. 2007;62:8–15.
- Ben Tkhayat R, et al. ERJ Open Res 2021;7:00332-2021.
- Berair R, et al. BMC Medicine. 2013; 11:145.
- Bernholm KF, et al. ERJ Open Res. 2018;4(4):00147-2017.
- Brightling CE, et al. N Eng J Med. 2021;385:1669–1679.
- Brightling CE, et al. N Engl J Med. 2002; 346:1699–1705.
- Brusselle GG and Koppelman GH. N Engl J Med. 2022;386(2):157–171.
- Busse WW. Allergol Int 2019;68:158–166.
- Cohen L, et al. Am J Respir Crit Care Med 2007;176:138–145.
- Cohn L. J Clin Invest. 2006;116(2):306–308.
- Collin M, et al. Immunology. 2018;154(1):3–20.
- Crespo-Lessmann A, et al. J Asthma Allergy. 2017;10:269–276.
- Davies AR, Hancox RJ. Curr Opin Pulm Med. 2013;19(1):60–65.
- Davies AR. Curr Opin Pulm Med. 2013:19(1):60–65.
- Davis JD and Wypych TP. Mucosal Immunology. 2021;14:978–990.
- Denton CP and Ong VH. J Scleroderma Relat Disord. 2017;2(2_suppl):S13–S19.
- Diver S, et al. Lancet Respir Med. 2021;9(11):1299–1312.
- Dunican EM, et al. J Clin Invest. 2018;128(3):997–1009.
- Erle DJ and Sheppard D. J Cell Biol. 2014;205(5):621–631.
- Escamilla-Gil JM, et al. Biomed Res Int. 2022. doi: 10.1155/2022/5753524.
- Gao H, et al. J Immunol Res. 2017;3743048.
- Gauvreau GM, et al. Expert Opin Ther Targets 2020;24(8):777–792.
- Global Strategy for Asthma Management and Prevention. 2023. Available from: www.ginasthma.org.
- Grygiel-Gorniak B, et al. Reumatologia.2017;55(6):298–305.
- Headley MB, et al. J Immunol. 2009;182(3):1641–1647.
- Heijink IH, et al. Clin Exp Allergy 2014;44:620–630.
- Heijink IH, et al. Allergy. 2020 Aug;75(8):1902–1917.
- Holgate ST. Immunol Rev. 2011;242(1):205–219.
- Huang C, et al. Front Pediatr. 2022;10:822043.

References [2/3]

- Hudey SN, et al. Curr Opin Immunol. 2020;66:123–128.
- Izuhara K, et al. Allergy Asthma Immunol Res. 2016;8(6):491–498.
- Izuhara K, et al. Allergy. 2019;74(11):2116–2128.
- Jack J, Bajaj T. Allergic bronchopulmonary aspergillosis. StatPearls [Internet]. StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK542329.
- Janeway CA, et al. Annu. Rev. Immunol. 2002. 20:197–216.
- Janeway ICA, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Glossary. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10759/.
- Jia Y, et al. Am J Respir Cell Mol Biol. 2016;55(5):675–683.
- Kato A, et al. J Immunol. 2007;179:1080–1087.
- Keun Kim C, Hagan JB. Ann Allergy Asthma Immunol. 2004;93(2):112–22.
- Kim H, et al. Allergy Asthma Clin Immunol 2017;13:48: doi: 10.1186/s13223-017-0219-4.
- Kim HY, et al. Nat Immunol. 2010;11(7):577–584.
- Kouzaki H, et al. J Immunol. 2009;183(2):1427–1434.
- Krystel-Whittemore M, et al. Front Immunol. 2016;6:620.
- Lambrecht BN and Hammad H. Nat Immunol. 2015;16(1):45–56.
- Licona-Limón P, et al. Nat Immunol. 2013;14(6):536–42.
- Likura M, et al. PLoS One. 2015;10(4):e0123584.
- Lombardi C, et al. Curr Res Immunol. 2022;3:42–53.
- Luo W, et al. Front. Immunol. 2022;13:974066.
- Marone G, et al. Front Pharmacol. 2019;10:1387.
- Matsumoto H. Respir Investig. 2020;58(3):144-54.
- McBrien CN and Menzies-Gow A. Front Med (Lausanne). 2017;4:93.
- MedLine Plus. Antineutrophil cytoplasmic antibodies (ANCA) test 2021. Available from: medlineplus.gov/lab-tests/antineutrophil-cytoplasmic-antibodies-anca-test.
- MedLine Plus. Antinuclear antibodies (ANA) test 2022. Available from: medlineplus.gov/lab-tests/ana-antinuclear-antibody-test.
- Monadi M, et al. Caspian J Intern Med. 2016 Winter;7(1):37–42.
- Moore VC. Breathe. 2012;8:232–240.
- Moore WC, et al. J Allergy Clin Immunol. 2011;28(2):328–336.
- Moral L, et al. Allergol Immunopathol (Madr). 2019;47(2):107–121.
- Mukherjee M, Nair P. Allergy Asthma Immunol Res. 2018;10(5):428–447.
- Ong LT, et al. EMJ Allergy Immunol. 2021;6(1):53–60.
- Oppenheimer J, et al. Ann Allergy Asthma Immunol. 2022;129:169–180.
- Page S, et al. Am J Physiol Lung Cell Mol Physiol. 2001;281: L1313–L1323.
- Pizzichini E, et al. Eur Respir J Suppl. 2002;37:9s–18s.
- Pizzichini E, et al. J Allergy Clin Immunol 1997;99(4):539–544.
- Porsbjerg CM, et al. Eur Respir J. 2020;56:2000260.
- Price D, Canonica GW. World Allergy Organ J 2020;13:100380.
- Ranu H, et al. Ulster Med J. 2011;80(2):84–90.
- Renauld CJ. J Clin Pathol. 2001;54:577–589.

References [3/3]

- Richardson MD and Page ID. Med Mycol. 2017;55(1):48–55.
- Roan F, et al. J Clin Invest. 2019;129(4):1441–1451.
- Salter BM, et al. J Leukoc Biol. 2019;106:889–901.
- Santus P, et al. J Clin Med. 2019;8(11):1955.
- Scott-Taylor TH. Immun Inflamm Dis. 2017;6(1):13–33.
- Severe Asthma Questionnaire. Available from: http://saq.org.uk/Download/SAQ.pdf.
- Shan L, et al. J Immunol. 2010;184:7134–7143.
- Shen F, Sergi C. Sputum analysis. StatPearls [Internet]. StatPearls Publishing; January 2023. www.ncbi.nlm.nih.gov/books/NBK563195.
- St George's Respiratory Questionnaire. Available from: https://www.thoracic.org/members/assemblies/assemblies/srn/questionaires/sgrq.php.
- Stone KD, et al. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S73–S80.
- Sverrild A, et al. Eur Respir J. 2022;59:2101296.
- Sverrild A, et al. J Allergy Clin Immunol. 2010;126:952–958.
- Sverrild A, et al. Respir Res. 2021;22:287.
- Tan DJ, et al. ERJ Open Res. 2021;7:00042–2020.
- Varricchi G, et al. Front Immunol. 2018;9:1595.
- Walford HH, et al. J Asthma Allergy. 2014;7:53–65.
- Walker C, et al. Curr Opin Pulm Med. 2012;18(1):42–47.
- Wan XC, et al. Immunol Allergy Clin North Am 2016:36(3);547–557.
- Wark PAB and Gibson PG. Thorax. 2006;61(10):909–915.
- West EE, et al. Drug Discov Today Dis Mech. 2012;9:3–4.
- Yoshimoto T. Front Immunol. 2018;9:716.
- Zhou B, et al. Nat Immunol 2005;6(10):1047–1053.