Clinical & Translational Immunology

Australian and New Zealand SOCIETY FOR IMMUNOLOGY INC.

Clinical & Translational Immunology 2022; e1417. doi: 10.1002/cti2.1417
www.wileyonlinelibrary.com/journal/cti

ORIGINAL ARTICLE

Increased mast cell activation in eosinophilic chronic obstructive pulmonary disease

Andrew Higham[1], Josiah Dungwa[2], Tuyet-Hang Pham[3], Christopher McCrae[3] & Dave Singh[1,2]

[1]Division of Immunology, Immunity to Infection and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester and Manchester University NHS Foundation Trust, Manchester, UK
[2]Medicines Evaluation Unit, The Langley Building, Manchester, UK
[3]Translational Science & Experimental Medicine, Early Respiratory & Immunology, Research and Early Development, AstraZeneca, One MedImmune Way, Gaithersburg, MD, USA

Correspondence
A Higham, Division of Immunology, Immunity to Infection and Respiratory Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester and Manchester University NHS Foundation Trust, 2nd Floor Research, Education and Research Building, Manchester, UK.
E-mail: andrew.higham@manchester.ac.uk

Received 25 April 2022;
Revised 8 August 2022;
Accepted 12 September 2022

doi: 10.1002/cti2.1417

Clinical & Translational Immunology
2022; **11**: e1417

Abstract

Objectives. A subset of chronic obstructive pulmonary disease (COPD) patients have increased numbers of airway eosinophils associated with elevated markers of T2 inflammation. This analysis focussed on mast cell counts and mast cell-related gene expression in COPD patients with higher vs lower eosinophil counts. **Methods.** We investigated gene expression of tryptase (*TPSAB1*), carboxypeptidase A3 (*CPA3*), chymase (*CMA1*) and two mast cell specific gene signatures; a bronchial biopsy signature (MC_{bb}) and an IgE signature (MC_{IgE}) using sputum cells and bronchial epithelial brushings. Gene expression analysis was conducted by RNA-sequencing. We also examined bronchial biopsy mast cell numbers by immunohistochemistry. **Results.** There was increased expression of *TPSAB1, CPA3* and MC_{bb} in eosinophil^{high} than in eosinophil^{low} COPD patients in sputum cells and bronchial epithelial brushings (fold change differences 1.21 and 1.28, respectively, $P < 0.01$). Mast cell gene expression was associated with markers of T2 and eosinophilic inflammation (*IL13, CLCA1, CST1, CCL26*, eosinophil counts in sputum and bronchial mucosa; rho = 0.4–0.8; $P < 0.05$). There was no difference in MC_{IgE} gene expression between groups. There was no difference in the total number of bronchial biopsy mast cells between groups. **Conclusion.** These results demonstrate that eosinophilic inflammation is associated with altered mast cell characteristics in COPD patients, implicating mast cells as a component of T2 inflammation present in a subset of COPD patients.

Keywords: eosinophils, epithelial cells, sputum, type 2 inflammation

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a highly heterogeneous and complex condition characterised by chronic airway inflammation and remodelling.[1] A subset of COPD patients have increased eosinophil infiltration into the lungs.[2,3] Blood eosinophil counts (BEC) are a biomarker of pulmonary eosinophil numbers, as these two parameters are correlated in COPD patients.[4–6]

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

Randomised controlled trials have shown that higher blood or sputum eosinophil counts are associated with greater clinical benefits of inhaled corticosteroids (ICS).[6,7] BEC are used in clinical practice to help guide ICS use towards COPD patients who are more likely to gain benefit.

Recent evidence has shown greater type 2 (T2) inflammation in the lungs of COPD patients with higher BEC, including increased levels of the eosinophil chemoattractant C-C motif chemokine ligand (CCL) 24, the eosinophil activator interleukin (IL)-5 and greater expression of the T2 genes *IL13, CCL26*, chloride channel accessory 1 (*CLCA1*) and cystatin SN (*CST1*).[5,8,9] Importantly, periostin (*POSTN*) and serpin family B member 2 (*SERPINB2*), which are well-known T2 genes in asthma, were not associated with BEC in COPD, highlighting that T2 inflammation in eosinophilic COPD and asthma are not identical.

Mast cells contain several granular proteases including tryptase, carboxypeptidase A3 and chymase. Anatomical location determines mast cell protease expression with compartmental differences observed in the lungs.[10] Mucosal mast cells are tryptase^{+}chymase^{-} (MC_T), whereas connective tissue mast cells are tryptase^{+}chymase^{+} (MC_{TC}). Mast cells are well known for their role in IgE-mediated allergic inflammation. However, mast cells also demonstrate IgE-independent functions including tissue repair and antimicrobial responses.[11] The role of mast cells in COPD is unclear with some, but not all, studies showing increased mast cell numbers in the lungs of COPD patients compared to controls.[12–14] These contrasting results may be because of patient selection, as higher sputum mast cell gene expression and tryptase protein levels have been observed in COPD patients with $> 3\%$ sputum eosinophils.[15,16] This suggests a role for mast cells in T2 inflammation in COPD.

Using single-cell RNA-sequencing of lung tissue, Vieira-Braga *et al*. identified mast cell genes present in asthma patients including genes encoding the proteases tryptase (*TPSAB1*), carboxypeptidase A3 (*CPA3*) and haematopoietic prostaglandin D synthase (*HPGDS*), the enzyme which produces prostaglandin D2.[17] In a subsequent study using these samples, Jiang *et al*. proposed an 11-gene mast cell signature with differential expression in asthma patients than in controls, which was then examined in bulk RNA-sequencing of bronchial biopsies from asthma patients. This mast cell bronchial biopsy [MC_{bb}] signature was related to the number of mast cells present and also appeared to be related to the activity of the cells as it was reduced in individuals using ICS.[18] Additionally, signatures which identify mast cell-specific genes involved in IgE-mediated inflammation have been identified following repeated activation of the mast cell IgE receptor, FcεRI (mast cell IgE [MC_{IgE}] signature).[19] These genes do not overlap with the MC_{bb} signature, and so may enable analysis of IgE-mediated vs non-IgE-mediated mast cell activation. Both the MC_{bb} and the MC_{IgE} signatures have been associated with eosinophilic inflammation in the sputum of severe asthma patients[20] but may represent different mechanisms of mast cell activation.

The aim of this analysis was to further investigate mast cell numbers and characteristics in COPD, and their associations with T2 inflammation. To characterise mast cells, common genes used to differentiate mast cell populations (*TPSAB1, CPA3 and CMA1*) were evaluated, as well as the gene expression signatures MC_{bb} and MC_{IgE} (Table 1). The effects of current smoking and ICS use were evaluated, and mast cell numbers assessed using immunohistochemistry/fluorescence. These analyses were performed using data and samples from a previously published study[5] comparing bronchial and sputum samples from COPD patients with higher vs lower BEC.

RESULTS

Study subjects

The clinical characteristics of the study participants have been previously reported[8] and are shown in Table 2. Patients with a prior asthma diagnosis or a positive skin prick test were excluded. The groups were generally well matched for clinical characteristics, with significant differences in sputum and BAL eosinophil percentage as expected ($P < 0.0001$ and $P = 0.02$, respectively).

Bronchial brushing gene expression

In bronchial epithelial brushings, *TPSAB1* and *CPA3* expressions were significantly higher in eosinophil^{high} than in eosinophil^{low} patients, with fold change differences of 1.21 and 1.28, respectively ($P = 0.002$ and $P < 0.0001$, respectively; Figure 1). *CMA1* expression showed a trend towards lower expression in eosinophil^{high} than in eosinophil^{low} COPD patients ($P = 0.07$).

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

Table 1. Individual genes used in mast cell signatures

Signature	Genes	Study
MC_{bb}	*TPSAB1, TPSD1, TESPA1, RGS13, SLC18A2, MS4A2, HPGDS, ADCYAP1, HDC, CPA3, TPSB2*	17
Repeated IgE (MC_{IgE})	*TMEM45B, EMR3, CH25H, LINC01272, DGAT2, MRC1, DPP4, DYSF, FPR2, MCEMP1, CCL18, TREM2, OLFM4, MX1, TGFBI, FOLR3, CYP4F3, S100A8, NUPR1, S100A12, VSTM1, HLADOA, LGMN, GLT1D1, CEBPE, S100A9, PADI4, CDA, F13A1, S100B*	19
Acute IgE (2 h IgE sensitisation 2 h FcεR1 activation)	*ATP8B4, BPI, CD33, CLC, CMA1, CPA3, CTSG, FCER1A, GZMB, HDC, HOXA1, HPGDS, IL18R1, IL1RL1, IL3, IL5, LINC00597, MARCH3, MS4A2, MS4A3, MYB, NOX3, NTRK1, P2RX1, RAB27B, RGS13, SLC12A8, STXBP6, TEC, TPSAB1*	20,35
Acute IgE (24 h IgE sensitisation 6 h FcεR1 activation)	*XIRP1, CRTAM, CNN1, AADAC, MIR3122, FASLG, CH25H, RAI2, LINC01010, CSF1, GZMB, CCL4L2, SERPINE2, TSPAN13, FHL2, CCL3L3, TGM2, LTBP4, HBEGF, EGR2, ASPHD1, CXCL8, SLC37A2, MIR221, KCNK5, ANGPTL4, SERPINE1, CCL7, DTL, MAGEB2, LINC01160, LRRC8B, CCL3, TNFSF4, CLCF1, LINC01433, SMOX, VGF, TIE1, GEM, RBFOX2, RAB7B, ZCCHC12, NRCAM, LRIG1, TRIB1, MIR103A2, MIR27A, DYRK3, PDGFA, CST6, KIAA0226L, NTN1, DGUOK-AS1, CTXN1, CCDC147-AS1, MAP1B, RGCC, GAL, SLC6A8, SPP1, PKIA, LRRC8C, NT5E, RRAD*	19,20
Acute IgE (overnight IgE sensitisation 24 h FcεR1 activation)	*CSF1, IL1R1, IL27RA, TNFRSF12A, CCL7, CCRL2, CXCL8, BL34, LIF, CD69, LAT, ADORA2A, CRIP1, HLA-DQB1, HKE2, NKG7, FCGR2B, PTGER2, CLECSF5, EBI2, TREM1, FCAR, TLR2, HIVEP1, FOSB, FBJ, EGR3, PHLDA2, FGFR1, EGR2, INSIG1, PDGFA, IER3, PDGFB, BTG2, TIEG, CNK, PBEF1, TNFAIP8, MLP, CRABP2, IRF2, RASAL1, FLRT2, SMARCD3, ARHE, KAL1, FLNB, CD151, ARF6, ALCAM, MAFF, BCL6, NFATC1, ATF3, NFKBIE, MYC, ELL2, TOP2A, GTF2H2, THBD, NR4A2, GEM, SPHK1, NDUFA7, HBEGF, JAG1, LDLR, MADH7, MALT1, SPRY2, DUSP6, MAP2K3, CREM, DUSP1, MAP3K14, FUT4, JUN, PSCDBP, FYN, PGGT1B, VRK2, TTK, ENC1, SLC16A6, HIST2H2AA, CYP3A4, HIST1H1C, HEC, STK17A, PELI1, KCNAB1, B4GALT4*	20,36
IL-33 activation	*IL13, EBI3, CD70, IL5, CXCL2, CXCL8, TNFRSF8, TNFRSF18, CCL3, IL6, IL3, WNT2B, TNF, KITLG, GDF11, IL1B, TNFSF11, TNFRSF1B, TNFSF14, TNFSF4, CCL3L3, GDF3, IL36G, WNT11, CXCL6, IL17D, EGF, TNFRSF17, AREG, NDP, EREG, UBD, FGD6, SSTR2, KIAA1324, CEP135, NFKBIZ, GADD45B, LIMS2, KLF5, ABTB2, ITGA1, P2RX5, NFKBIA, C15orf48, LARGE2, IL2RG, RMI1, KCNH2, RIPOR3, CFLAR, HIVEP2, ZC3H12A, MIIP, RGS9, TTC39C, PMAIP1, PTX3, TLR2, RANBP9, NAPB, TNIP1, EGLN1, METAP2, ASNS, IER3, AMMECR1, MPZL1, FLVCR1, MT2A, PIM2, TMEM64, VWA8, PEAK1, ADIPOR2, TEX30, HAPLN3, KISS1R, ADAM8, TAP2, NFKB1, SLC4A7, CDC42EP2, SLC25A45, MMP9, DES*	20,37
LPS activation	*GNG4, IRF4, CSF2, IR155HG, IL6, MIR212, IL1RN, LINC01215, LOC731424, GGT5, CCL3L1, FFAR2, F3, PPBP, MIR146A, IL7R, TARP, MIR222, TM4SF1, MAOA, CCL4L1, MAMLD1, STEAP4, C3, TNFAIP6, STAT4, EBI3, IGSF6, IL2RA, NFKBIA, AQP9, ABTB2, IRAK2, EMR1, SNORA66, NFKB1, REL, CCL20, RGS9, MFSD2A, CXCL1, TNIP1, C17orf96, NFKB2, TNFRSF18, PXYLP1, LY6G6C, HILPDA, C15orf48, DUSP2, CXCL2, CYP27B1, DUSP5, PTX3, MSC, TNFSF14, GCH1, TMEM88, SLAMF1, EMP1, CLEC4A, RASGRP1, MAP3K8, FLVCR2, EDIL3, MREG, AQP2, CD40, BATF3, CD274, TNFRSF8, SLAMF7,*	20,38

(Continues)

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

Table 1. Continued.

Signature	Genes	Study
	METTL1, *IL15RA*, *CCL24*, *IL1B*, *RNF144B*, *SH3RF2*, *SERPINB2*, *CISH*, *POU2F2*, *SCARF1*, *CCDC147*, *PRG2*, *PEAK1*, *TNIP3*, *GPR84*, *CXCL5*, *CD83*, *LPL*, *TRIP10*, *PPARG*, *SNORA61*, *GSAP*, *NLRP3*, *TNFAIP3*, *ECE1*, *ATP9A*, *PDLIM4*, *SDC4*, *RELB*, *APOL1*, *SNORA6*, *SERPINA1*, *ELOVL7*, *TNFAIP2*, *BCL2A1*, *GBP2*, *FAS*, *SLC2A6*, *ECEL1*, *SPATA13*, *STON2*, *CKB*, *PIM2*, *FPR2*, *SGPP2*, *THBS1*, *RSAD2*, *GPC1*, *FGL2*, *CA12*, *ETV3*, *SOCS2*, *IL10RA*, *ENTPD7*, *BACH2*, *DPP4*, *LUCAT1*, *SLCO4A1*, *PRDX1*, *RASAL2*, *NINJ1*, *ESPL1*, *RAB38*, *OLR1*, *TMEM163*, *BATF*, *CLEC5A*, *ARHGAP31*, *ICAM1*, *STAMBPL1*, *RASSF4*	
IFN-γ activation	*MARKS*, *MT1E*, *MUC1*, *SDCCAG8*, *SGPL1*, *ITGA1*, *MYB*, *CD40*, *MSL3*, *CCL8*, *AP1G1*, *PDZD2*, *APOL1*, *P2RX5*, *B3GNT7*, *CLIP1*, *SLC2A6*, *KYNU*, *TIMP1*, *CYB5A*, *SLC11A2*, *EZH2*, *TNFSF10*, *AGT*, *TPBG*, *ARG2*, *CEBPD*, *CD48*, *FSTL3*, *PRPSAP2*, *FCGR2A*, *JAK1*, *IGF2R*, *PLSCR1*, *TNFAIP2*, *ALCAM*, *EHD1*, *ABCG4*, *BID*, *G0S2*, *CRYBG1*, *MAOA*, *NOP16*, *METAP2*, *NFKB2*, *PLA2G4A*, *CSF2*, *CFLAR*, *AMACR*, *CREB3*, *GADD45B*, *CITED2*, *TANK*, *CXCL8*, *MT1H*, *TMEM123*, *MT2A*, *IPO5*, *TTC39A*, *ANXA2*, *EIF4A3*, *ADAP1*, *CYLD*, *DLEU2*, *RBM7*, *TMEM165*, *TNFAIP3*, *ICAM1*, *GBP2*, *GCH1*, *BUB3*, *SH3BP5*, *STAG1*, *HSPA13*, *ACSL1*, *SLC7A1*, *CHST7*, *NEURL1*, *MAMLD1*, *CCL3*, *MAP3K8*, *BCL2A1*, *ZSCAN21*, *TSFM*, *H2AC18*, *EIF5B*, *EIF5A*, *DCBLD2*, *EMD*, *CRY1*, *HLA-DRB4*, *ARL6IP1*, *TASP1*, *SINHCAF*, *UBIAD1*, *CDK17*, *GLS*, *RAB31*, *RBM8A*, *CHORDC1*, *PELO*, *SMARCA5*, *KLF10*, *DNAJB4*, *GSPT1*, *ZNF222*, *HMGB2*, *EIF5*, *PPP2CA*, *CD44*, *MKLN1*, *IL7R*, *RCE1*, *FGL2*, *RRAD*, *ATG12*, *ISG20*, *CXCL11*, *HLA-DRA*, *STAT1*, *CD74*, *MX1*, *HLA-DQA1*, *IFIT1*, *HLA-DQB1*, *IL3RA*, *AKR1C3*, *RSAD2*, *CBR3*, *TAP1*, *ISG15*, *SERPING1*, *HBD*, *SECTM1*, *HLADPB1*, *DHX58*, *OAS1*, *OAS3*, *WARS1*, *CXCL10*, *IFIH1*, *HBB*, *HLADPA1*, *TRIM14*, *IFI44*, *DDX58*, *FOG2*, *LGALS3BP*, *PSMB9*, *IFI35*, *IFIT4*, *HCP5*, *USP18*, *HLA-DOB*, *TRAFD1*, *SAMHD1*, *HLA-DRB5*, *HLA-DMA*, *OASL*, *DOCK9*, *HLA-DRB3*, *FKBP11*, *AVIL*, *PSME2*, *POLA2*, *IRF7*, *LY6E*, *NOD2*, *CTSS*, *PLAAT4*, *BTN3A3*, *C4B*, *HLADRB1*, *XAF1*, *HLA-DMB*, *OAS2*, *TRIM58*, *PSMB8*, *BST2*, *TAP2*, *ZC3HAV1*, *UBE2L6*, *RCBTB2*, *IFITM1*, *CD38*, *HLA-G*, *CASP1*, *NKX3-1*, *SQOR*, *NBN*, *FOSB*, *IRF1*, *TRIM22*, *TNIP2*, *TNFAIP6*, *CEP135*, *RTP4*, *TKT*, *IFI6*, *MXI1*, *SNN*, *PSME1*, *MT1G*, *KLF4*, *FADD*, *MDS019*, *POLR3D*, *DOP1A*, *RPS6KC1*, *ADGRE5*, *TYMP*, *OFD1*, *IFNGR2*, *CASP10*, *PSMB10*, *RAB33A*, *IL13*, *DNAJA1*, *NDP52*, *TIA1*, *DUSP1*, *STK39*, *SP100*, *BAZ1A*, *EIF1AY*, *TNFAIP8*, *SOCS1*, *RNF114*, *MAP3K7*, *ARHGAP25*, *IRLB*, *ATF3*	[20,38]

The MC_{bb} signature was significantly higher in eosinophil^{high} than in eosinophil^{low} patients ($P = 0.0003$; Figure 1), whilst there was no difference between groups for the MC_{IgE} signature ($P = 0.3$; Figure 1). To ensure *TPSAB1* and *CPA3* expressions were not driving the MC_{bb} signature results, we performed a subanalysis that removed these genes; the differences between the groups remained significant ($P = 0.0005$; data not shown).

There were no differences in the expression of other mast cell signatures between eosinophil^{high} and eosinophil^{low} COPD patients (Supplementary figure 1), apart from an acute IgE activation signature (2 h IgE sensitisation 2 h FcεR1 activation: $P = 0.0003$).

We analysed associations between T2 and eosinophil-related markers and the MC_{bb} signature. The MC_{bb} signature was significantly associated with the COPD T2 signature (rho = 0.6; $P = 0.0001$; Figure 2), and the individual genes within the T2 signature (data shown in Supplementary figure 2), BEC (rho = 0.6; $P = 0.0004$), sputum eosinophil counts (rho = 0.4;

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

Table 2. Clinical characteristics of the study population

	Eosinophil^{low}	Eosinophil^{high}	*P*-value
Age (year)	62 ± 6	62 ± 4	0.7
Gender: Male (%)	57	70	0.5
BMI (kg/m^2)	29 (18–34)	25 (19–32)	0.1
Current smokers (%)	43	60	0.4
Pack-years history	42 ± 15	38 ± 14	0.4
ICS use (%)	76	55	0.2
Post bronchodilator FEV_1 (L)	1.8 ± 0.4	1.9 ± 0.4	0.45
Post bronchodilator FEV_1 (% predicted)	62 ± 11	66 ± 11	0.4
Post bronchodilator FVC (L)	3.7 ± 1.2	3.6 ± 0.9	0.8
Post bronchodilator FEV_1/FVC ratio	50 ± 10	53 ± 7	0.3
Reversibility (mL)	190 ± 183	220 ± 154	0.6
$FeNO_{50}$ (ppm)	15 (5–30)	21 (2–61)	0.2
Atopy (% positive)	0	0	N/A
Total SGRQ	42 ± 15	37 ± 21	0.4
mMRC	1 (1–4)	1 (0–4)	0.1
CAT	17 (5–35)	17 (4–32)	0.5
Exacerbation rate, 12 months prior	0 (0–3)	0 (0–3)	0.7
Blood eosinophils (cells μL^{-1})	100 (60–140)	410 (280–890)	< 0.001
Sputum eosinophils (%)	0.5 (0.5–2.5)	4.5 (0.25–70)	< 0.001
BAL eosinophils (%)	0 (0–3.25)	0.75 (0–8)	0.02

Data are presented as %, mean ± standard deviation, or median (range).

BMI, body mass index; CAT, COPD Assessment Test; ICS, inhaled corticosteroids; $FeNO_{50}$, fractional exhaled nitric oxide at 50 mL s^{-1} flow rate; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; mMRC, modified Medical Research Council; PPM, parts per million; SGRQ, St George's Respiratory Questionnaire.

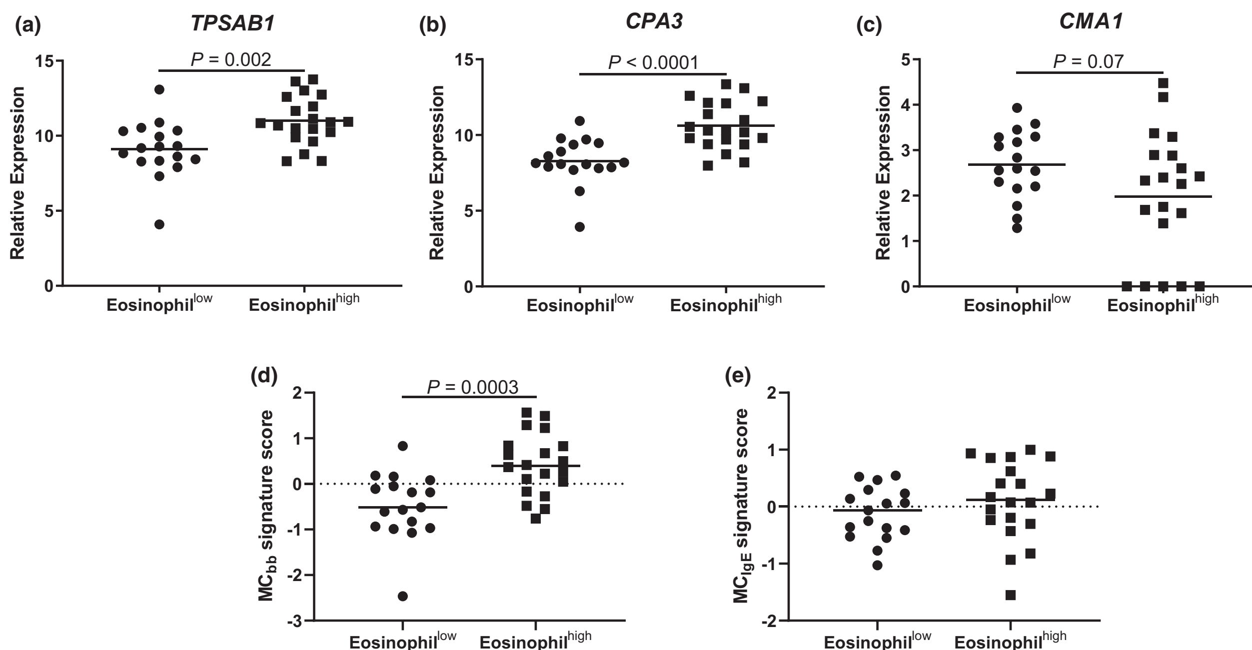


Figure 1. Bronchial brushing mast cell gene expression in eosinophil^{low} and eosinophil^{high} chronic obstructive pulmonary disease (COPD) patients. RNA-sequencing was used to examine the expression of **(a)** *TPSAB1*, **(b)** *CPA3*, **(c)** *CMA1*, **(d)** MC_{bb} signature and **(e)** MC_{IgE} signature in n = 17 eosinophil^{low} and n = 20 eosinophil^{high} COPD patients. Data are presented as individual values where the black horizontal line represents the mean **(a–c)** or median **(d** and **e)**.

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

P = 0.04) and tissue eosinophil counts (rho = 0.6; P = 0.0009; Figure 2). There were no associations with *IL5* or FeNO (Supplementary figure 3; rho = −0.07; P = 0.7 and rho = 0.3 and P = 0.08, respectively). There was no association between the COPD T2 signature, eosinophil counts and the MC_{IgE} signature (Supplementary figure 4). *TPSAB1* and *CPA3* expressions and the MC_{bb} signature were significantly lower in ICS users (n = 25) than in non-users (n = 12; Figure 3; $P < 0.01$ for all comparisons). In the eosinophil^{high} group, the MC_{bb} signature was lower in ICS users (n = 11) vs non-users (n = 9), and there was a trend for reduced *TPSAB1* and *CPA3* expressions but this did not reach statistical significance (Supplementary figure 5; P = 0.05–0.1). The lower sample size in the eosinophil^{low} group (ICS users and non-users; n = 14 and n = 3, respectively) prevented comparisons. There were no differences between current smokers and ex-smokers (Supplementary figure 6).

Mast cell counts in bronchial biopsies

Mast cell numbers in bronchial biopsies were examined by dual immunofluorescence. There was no difference in the total number of mast cells ($M_T + M_{TC}$), the number of M_T or the number of M_{TC} in the subepithelium of eosinophil^{high} compared with eosinophil^{low} COPD patients (Figure 4). In ICS users (n = 16), the total number of mast cells, the number of M_T and the number of M_{TC} were significantly lower than in non-users (n = 7; Figure 4; P = 0.02, P = 0.02 and P = 0.04, respectively). In eosinophil^{high} patients, there was a numerical reduction in mast cell numbers in ICS users (n = 7) than in non-users (n = 6) but this did not reach statistical significance (Supplementary figure 7; $P > 0.05$). There were no differences due to current smoking (Supplementary Figure 7). The number of M_T was significantly higher than M_{TC} in eosinophil^{high} and eosinophil^{low} groups (P = 0.007 and P = 0.05, respectively).

The number of biopsies with intact epithelium available for new experiments was low (n = 9 eosinophil^{high} vs n = 4 eosinophil^{low}), making it difficult to compare intra-epithelial mast cell counts with these samples. We therefore counted intra-epithelial mast cells using slides already stained with single label tryptase from the original publication, where only subepithelial mast cell counts were reported previously.[5] In 15 eosinophil^{high} vs 14 eosinophil^{low} patients, there was no difference in the number of intra-epithelial mast cell numbers when normalised to epithelial thickness (Figure 4; P = 0.6) or basement membrane length (Supplementary figure 7; P = 0.8).

There was no difference in epithelial thickness or PAS$^+$ cells between eosinophil^{high} vs eosinophil^{low} patients (Supplementary figure 8).

Sputum cell gene expression

In sputum, *TPSAB1* and *CPA3* expressions trended higher in eosinophil^{high} than in eosinophil^{low} COPD patients (Supplementary figures 9; Figure 5; P = 0.07). The expression of *CMA1* was not different between groups (Supplementary figure 9; P = 0.9). The MC_{bb} signature was significantly higher in eosinophil^{high} than in eosinophil^{low} COPD patients (Figure 5; P = 0.004) and significantly associated with the COPD T2 signature (rho = 0.6 = P = 0.001; Figure 5) and the individual genes within the T2 signature (Supplementary figure 10). The MC_{bb} signature was also significantly associated with blood and sputum eosinophil counts (Figure 5; rho = 0.5; P = 0.009; rho = 0.8; $P < 0.0001$). There was a significant association with *IL5* gene expression but not FeNO (Supplementary figure 11; rho = 0.8; $P < 0.0001$ and rho = 0.3; P = 0.2, respectively). The sputum MC_{bb} signature was significantly associated with the bronchial brushing MC_{bb} signature (Supplementary figure 11; rho = 0.5; P = 0.01). When *TPSAB1* and *CPA3* were removed from the MC_{bb} signature, expression remained significantly higher in the eosinophil^{high} patients than in the eosinophil^{low} patients (P = 0.006; data not shown).

There was no difference between groups for the MC_{IgE} signature (P = 0.7; Supplementary figure 12), and there was no correlation between MC_{IgE} signature and the COPD T2 signature or eosinophil counts (Supplementary figure 12). We found no differences in the expression of other mast cell signatures between eosinophil^{high} and eosinophil^{low} COPD patients (Supplementary figure 13), apart from an acute IgE activation signature (2 h IgE sensitisation 2 h FcεR1 activation: P = 0.01). There was no difference between ICS users and non-users (Supplementary figure 14) and current and ex-smokers (Supplementary figure 15) for individual sputum gene expression or the sputum MC_{bb} signature.

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

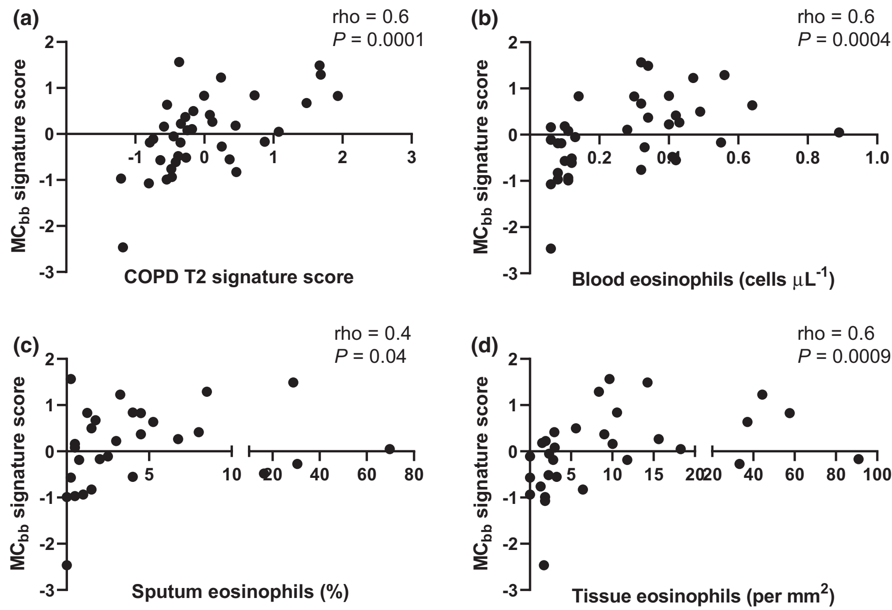


Figure 2. Correlations between bronchial brushing mast cell gene expression and T2 biomarkers. RNA-sequencing was used to examine correlations between the MC_{bb} signature and **(a)** chronic obstructive pulmonary disease (COPD) T2 signature, **(b)** blood eosinophils, **(c)** sputum eosinophils and **(d)** bronchial biopsy eosinophilsin n = 17 eosinophil^{low} and n = 20 eosinophil^{high} COPD patients. Data were analysed by Spearman's correlation.

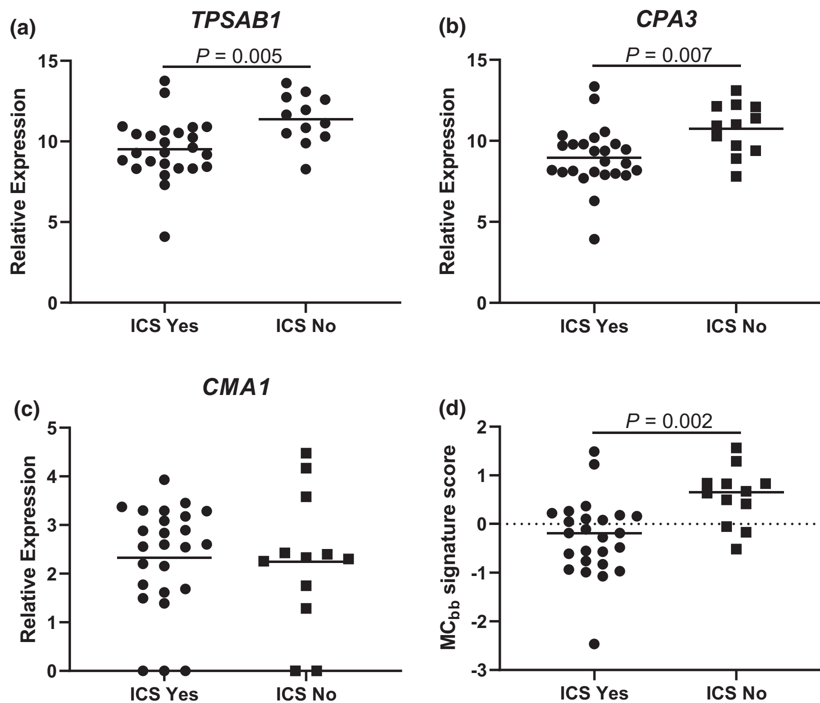


Figure 3. Bronchial brushing mast cell gene expression in inhaled corticosteroids (ICS) users and non-users. RNA-sequencing was used to examine the expression of **(a)** *TPSAB1*, **(b)** *CPA3*, **(c)** *CMA1*, **(d)** MC_{bb} signature and **(e)** MC_{IgE} signature in n = 25 ICS users (ICS Yes) and n = 12 non-users (ICS No). Data are presented as individual values where the black horizontal line represents the mean **(a–c)** or median **(d)**.

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

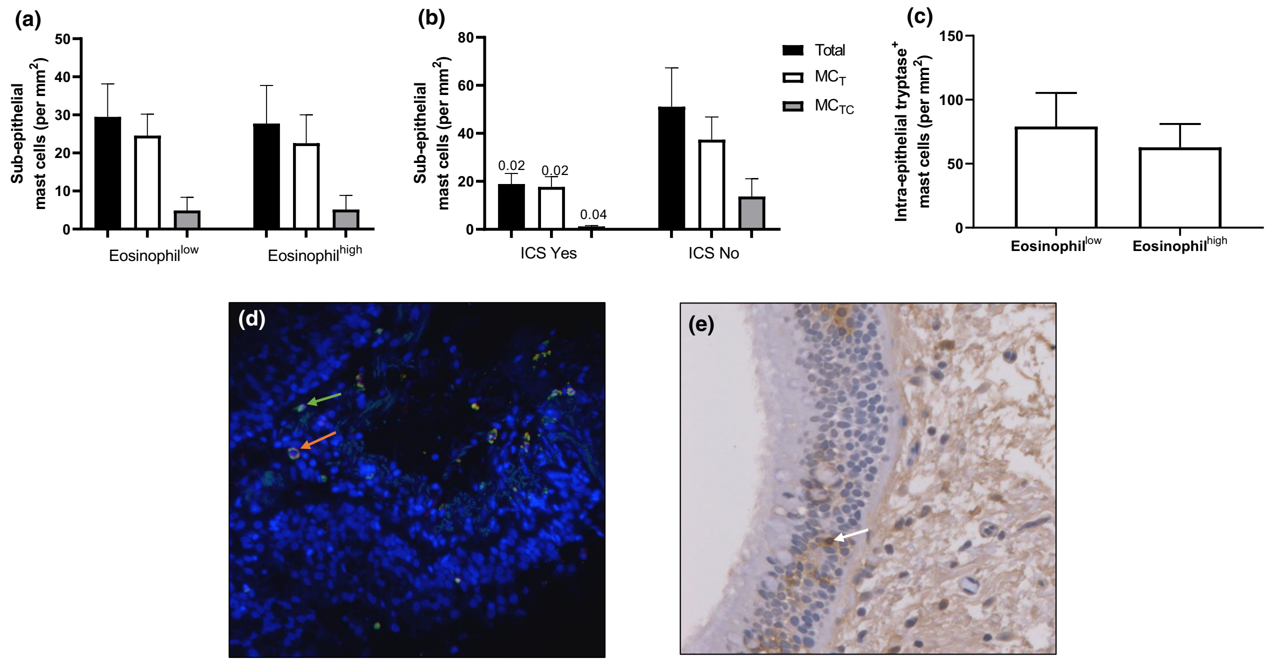


Figure 4. Mast cell quantification in bronchial biopsies. The number of subepithelial tryptase+ (M_T), tryptase+chymase+ (M_{TC}) and the total number of mast cells ($M_T + M_{TC}$) were quantified by immunofluorescence **(a, b)** and the number of intra-epithelial tryptase+ mast cells were quantified by immunohistochemistry **(c)**. Representative images of immunofluorescence where green and orange arrows indicate M_T and M_{TC} cells respectively **(d)** and immunohistochemistry where white arrows indicate tryptase-positive mast cells **(e)**. Comparisons were made between eosinophil^{low} vs eosinophil^{high} patients **(a)** n = 10 vs *n* = 13; **(c)** *n* = 14 vs *n* = 15; ICS users vs non-users **(b)** *n* = 16 vs *n* = 7. *P*-values in **b** signify differences between the same mast cell populations in ICS Yes vs ICS No groups.

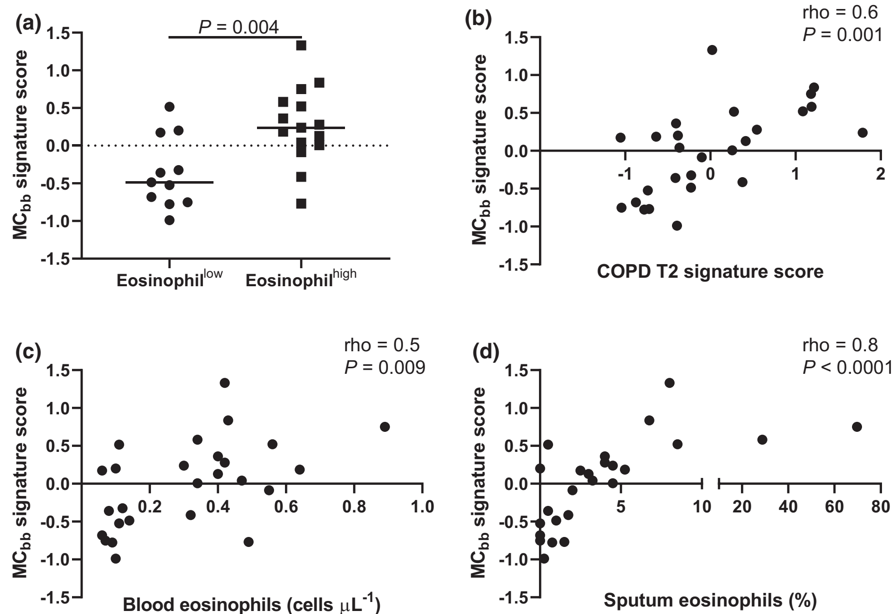


Figure 5. Sputum mast cell gene expression in eosinophil^{low} and eosinophil^{high} COPD patients. RNA-sequencing was used to examine the expression of **(a)** MC_{bb} signature and correlations between the MC_{bb} signature and **(b)** COPD T2 signature, **(c)** blood eosinophils and **(d)** sputum eosinophils in *n* = 11 eosinophil^{low} and *n* = 15 eosinophil^{high} COPD patients. Data are presented as individual values where the black horizontal line represents the median **(a)**. Data are analysed by Spearman's correlation **(b–d)**.

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

DISCUSSION

Mast cell gene expression, namely *TPSAB1*, *CPA3* and a mast cell-specific MC_{bb} signature, was increased in eosinophil^{high} compared to eosinophil^{low} COPD patients. These findings were consistent in both bronchial epithelial brushings and sputum samples, with the MC_{bb} signature also being correlated with T2 biomarkers. Bronchial mast cell numbers did not differ between groups. These findings suggest that the phenotypic characteristics of mast cells are different in eosinophil^{high} vs eosinophil^{low} COPD patients.

Studies in asthma patients have shown that mast cell numbers and mast cell gene expression do not consistently correlate.[18,20] Berry *et al*. reported no difference in the number of submucosal tryptase^{+} mast cells in eosinophilic vs non-eosinophilic asthma patients.[21] We observed altered mast cell gene expression (*TPSAB1, CPA3* and MC_{bb}) in eosinophil^{high} compared with eosinophil^{low} COPD patients, but no differences in mast cell numbers. We interpret these COPD results as showing altered mast cell phenotype despite no changes to intra- and subepithelial mast cell numbers. It is possible that our results also indicate increased mast cell activity in eosinophil^{high} COPD patients, although other measures of mast cell activation would be needed to properly investigate this. The association between mast cell gene expression and both the T2 signature (*IL13, CLCA1, CST1* and CCL26) and eosinophilic airway inflammation (measured in sputum and bronchial mucosa) indicates that altered mast cell characteristics are part of a T2/eosinophilic profile in a subset of COPD patients.

We observed decreased mast cell gene expression and lower mast cell numbers in ICS users than in non-users. Lower mast cell numbers associated with ICS use have also been reported in both asthma and COPD studies.[18,22–24] The reduction in mast cell numbers with ICS use provides evidence of the sensitivity of our immunohistochemistry stains to detect changes in subepithelial mast cell numbers, and therefore indirectly further supports the robustness of the finding of no difference in total mast cell numbers in eosinophil^{high} vs eosinophil^{low} COPD patients.

There is previous evidence from sputum analysis of greater mast cell activation in COPD patients with $> 3\%$ vs $< 3\%$ eosinophils, with higher levels of tryptase measured.[16] We analysed the bronchial epithelium, in addition to sputum, to enable detailed analysis of mast cell characteristics in COPD. Immunohistochemistry indicated a predominance of mucosal (MC_T) over connective tissue mast cells (MC_{TC}), compatible with previous findings in the endobronchial biospies of asthma patients.[25] There was a similar pattern for gene expression analysis where *TPSAB1* (tryptase) was higher than *CMA1* (chymase) expression. Furthermore, the increased gene expression of *TPSAB1* and *CPA3*, with no increase in *CMA1* expression, in eosinophil^{high} vs eosinophil^{low} COPD patients, is similar to previous observations in asthma patients with increased eosinophil counts.[25,26] Eosinophilic inflammation in both asthma and COPD therefore appears to be associated with a predominance of the MC_T phenotype.

The MC_{IgE} signature did not differ between groups. This signature is derived from a repeated IgE exposure mast cell activation model, and we therefore conclude that chronic IgE-mediated mast cell activation is not involved in eosinophilic inflammation in COPD. Of note, the groups in this study had similar serum IgE levels, and only non-atopic individuals were included. We also examined other *in vitro* mast cell signatures described by Tiotiu *et al*.[20] which examined acute IgE stimulation (three signatures), IL-33 stimulation, LPS stimulation and IFN-γ stimulation of mast cells. There were no differences in the expression of these signatures between eosinophil^{high} and eosinophil^{low} patients, apart from an acute IgE signature (2 h IgE sensitisation 2 h FcεR1 activation) which contains several genes that overlap with the MC_{bb} signature (*CPA3, HDC, HPGDS, RGS13 and TPSAB1*) and also includes *CLC* which is also strongly expressed by eosinophils. However, the general lack of positive results from the Tiotiu *et al*.[20] signatures, which were primarily used to investigate asthma, likely reflect different pathophysiological processes in COPD.

It has been reported that chymase-only positive mast cells are present in the bronchi,[27] although none were observed in the current study. Our findings are in agreement with another study in COPD patients.[14] It should also be noted that the type of tissue fixative used can influence mast cell identification in the lungs; Kleinjan *et al*.[28] observed that the percentage of chymase-only mast cells in the lung parenchyma of tissue fixed with acetone was 1%, whereas in tissue fixed with Carnoy's fluid, chymase-only mast cells were

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

undetectable. Overall, it appears that the relative numbers of these cells are small and that methodology influences successful detection.

It has been reported that mucosal mast cell numbers in the small airways of COPD GOLD stage 1–3 patients are similar to controls, but there are reduced numbers in GOLD 4 patients.[14] We included GOLD 1–3 patients and found differences in mast cell gene expression associated with eosinophil counts, highlighting mast cell heterogeneity within COPD patients.

Our study has limitations. We used bulk RNA-sequencing data to investigate mast cell signatures. However, the mast cell signatures used were developed from single-cell analysis and further validated in mixed-cell samples.[18] It will be important to confirm our findings in COPD using single-cell RNA-sequencing. Whilst our sample size is modest, the well-phenotyped nature of the subjects enables evaluation of eosinophil^{high} vs eosinophil^{low} individuals. Due to sample availability, we were unable to measure extracellular markers of mast cell activation, for example prostaglandin D2 and tryptase. We conducted multiple testing (correlations) which can increase the number of type 1 errors. However, these correlations were exploratory in nature to supplement the main analyses. A healthy control group analysis may aid interpretation of the level of mast cell activation, although it has been reported that mast cell numbers in healthy subjects are very low.[18] Finally, the aim of our study was to investigate mast cells in eosinophilic COPD; other cell types may also be relevant (e.g. Th2 cells), which could be the focus of future investigations.

In conclusion, the data reported here show altered mast cell phenotype and characteristics in COPD patients with eosinophilic inflammation. There is a growing understanding of the nature of T2 and eosinophilic inflammation in COPD, which represents a potential target for pharmacological treatment.[29,30] Our findings help understand the complexity of T2 and eosinophilic inflammation in COPD, which appears to encompass changes in mast cell characteristics.

METHODS

Study subjects

Chronic obstructive pulmonary disease patients aged > 40 with a smoking history of > 10 pack-years, a postbronchodilator forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) ratio < 0.7, and with no history of asthma were recruited. Patients receiving oral corticosteroids or antibiotics within 6 weeks of the study were excluded. Atopy was determined by a positive skin prick test against house dust mite extract, cat dander or grass pollen. Here, we include samples from $n = 20$ eosinophil^{high} (> 250 cells μL^{-1}) and $n = 17$ eosinophil^{low} (< 150 cells μL^{-1}) COPD patients because of sample availability. This study was conducted in accordance with the Declaration of Helsinki 1975. Sample collection was approved by the local research ethics committees (REC): South Manchester REC 06/Q1403/156 (brushings), Tameside and Glossop local REC 05/Q1402/41 (sputum) and NRES Committee North West–Preston 10/H1016/25 (blood).

Sputum and bronchoscopy samples

Sputum was induced using 3%, 4% and 5% saline, inhaled in sequence for 5 min, up to 15 min via an ultrasonic nebuliser (EASYneb II, Flaemnouva, Italy) and processed as previously reported.[31] To minimise contamination of saliva, all subjects were instructed to thoroughly rinse their mouth with distilled water and perform coughing prior to sputum expectoration. Sputum plugs were isolated from the saliva component, combined proportionately with phosphate buffered saline (PBS) and vortexed for 10 s, rocked for 15 min and centrifuged (790 *g* for 10 min at 4°C). PBS supernatants were removed and 0.2% dithiothreitol (DTT) was added and the suspension was vortexed for 10 s, rocked for 15 min and filtered using a 48 μm filter (Sefar Ltd, Manchester, UK). The suspension was centrifuged (790 *g* for 10 min at 4°C), and DTT supernatants were removed. The cell pellet was lysed in RLT buffer (Qiagen, Crawley, UK) plus β-mercaptoethanol added according to the manufacturer's instructions prior to RNA extraction. Before lysing, a small number of cells were removed to prepare cytospins. Slides were air dried for 30 min and then fixed in methanol for 10 min before staining with RapiDiff (Triangle, Skelmersdale, UK) for differential cell counting.

Some patients included in this study have been used in previous publications.[5,8]

Bronchoscopy was performed after the subjects had been sedated as previously described.[31] BAL was collected from the right and/or left upper lobe. The bronchoscope was wedged in the bronchus and a maximum of 4 × 60 mL aliquots of prewarmed sterile 0.9% NaCl solution were instilled per lobe. The aspirated fluid was stored on ice before filtration (100 μm filter, Becton Dickenson, Oxford, UK). The filtrate was centrifuged (400 *g* for 10 min at 4°C), and the BAL fluid removed. The cell pellet was lysed in RLT buffer plus β-mercaptoethanol prior to RNA extraction. Bronchial brushings were collected in bronchial epithelial basal medium (Lonza, Basel, Switzerland) and stored on ice before centrifugation (400 *g* for 10 min at 4°C). The cell pellet was lysed in RLT buffer plus β-mercaptoethanol prior to RNA extraction. Endobronchial biopsies were collected from airway generations 2–5 using 2-mm radial jaw biopsy forceps (Boston Scientific, Hemel Hempstead, UK) and immediately fixed in 4% neutral buffered formalin, before being processed and embedded in paraffin.

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

RNA extraction and cDNA analysis

Total RNA was extracted from sputum cells and bronchial epithelial brushings using ZR RNA MicroPrep kit (Zymo Research, Orange, CA, USA) and RNA-seq libraries were prepared using TruSeq Stranded mRNA Prep kit (Illumina, San Diego, CA, USA) per manufacturers' protocols. To confirm RNA quality, RIN scores ≥ 7 were evaluated using the 2100 Bioanalyzer system (Agilent, Santa Clara, CA, USA). Paired-end sequencing (75 base pairs per end) with sequencing depth at 80 million reads was performed on the HiSeq2000 platform (Illumina) to generate FASTQ files. These were aligned to human genome (version HG19) using HiSAT2 (John Hopkin's University, Baltimore, USA) and SAMtools (Genome Research Limited, Cambridge, UK). Normalised read counts were generated per transcript using DESeq2.[32] Read counts were then transformed to $\log_2$ scale (after adding 1 to account for zero read counts).

Gene expression analysis

All the gene expression analysis was conducted using bulk RNA-sequencing data generated from bronchial brushings or sputum cells. We compared the individual expression of common mast cell genes (*TPSAB1, CPA3* and *CMA1*) along with mast cell signatures generated from asthma bronchial biopsies (MC_{bb} signature) and cord blood derived mast cells sensitised with IgE for 24 h followed by repeated activation of FcεRI with anti-IgE for 2 weeks (MC_{IgE} signature).[17,20] We also included other mast cell signatures including acute IgE activation (three signatures), IL-33 activation, lipopolysaccharide (LPS) activation and interferon (IFN)-γ activation as per Tiotiu *et al*. Full details of the genes included are shown in Table 1.

We correlated the expression of mast cell signatures (MC_{bb} signature and MC_{IgE} signature) with our previously validated COPD T2 signature (*IL13, CLCA1, CST1* and *CCL26*) which was elevated in the sputum and bronchial brushings of COPD patients with higher BEC.[8] We also correlated mast cell signatures with *IL5* gene expression and FeNO. The gene expression scores for each signature were calculated as per Bhakta *et al*. and Southworth *et al*.[33,34]

Immunohistochemistry and immunofluorescence

All antibodies were validated to determine the optimal conditions prior to sample staining using tonsil as a positive control tissue. The optimal antigen retrieval method was selected from either; citrate buffer pH 6, EDTA pH 8, tris EDTA buffer pH 9, no retrieval or pepsin enzyme digestion. This was followed by optimal antibody concentration and finally testing the specificity of the antibody using an isotype control and omission of the primary antibody.

Immunohistochemistry for tryptase^{+} mast cells was previously reported.[5] Briefly, biopsies were cut into 3-μm sections, dewaxed and rehydrated before heat-induced antigen retrieval in a citrate pH 6 buffer. Mast cells were identified using mouse monoclonal anti-tryptase (clone AA1, Dako, Stockport, UK). The immune reaction was detected using the Imm-PRESS Excel Anti-mouse kit (Vector, Peterborough, UK). Bronchial biopsies were imaged and analysed using ImagePro Plus-6.0 at a magnification of x200. The number of tryptase-positive cells were quantified in the bronchial epithelium by a single blinded observer and expressed per mm^2.

Double-immunofluorescence staining for mast cell tryptase and mast cell chymase was conducted on 3 μm bronchial biopsy sections. Deparaffinisation and antigen retrieval were carried out for 20 min at 97C in a PT-Module (Thermo Fisher, Runcorn, UK) using the Thermo Dewax and HIER Buffer M (Thermo Fisher). Following incubation with protein blocking solution (Abcam, Cambridge, UK), the slides were incubated in mouse monoclonal antitryptase (clone AA1, Dako 1:200) and mouse monoclonal antichymase (Clone CC1, Abcam 1:100) antibodies, stained sequentially. To increase the sensitivity of the staining, secondary antibodies were employed in the form of goat anti-mouse IgG followed by the detection of the primary–secondary antibody reaction using donkey anti-goat IgG H&L (Alexa Fluor® 488) and donkey anti-goat IgG H&L (Alexa Fluor® 568) The omission of primary antibodies was used as negative controls. Finally, Hoescht 33258 (Abcam) was added to each sample for nuclear staining. Digital micrographs were obtained using a Nikon Eclipse 80i microscope (Nikon UK Ltd, Surrey, UK) equipped with a QI imaging digital camera and analysed using ImagePro Plus-6.0 at a magnification of x200. The number of tryptase and/or chymase-positive cells was quantified in the bronchial epithelium and subepithelial layer by a single blinded observer and expressed mm^2. Counts were quality control checked with an interuser agreement of $< 10\%$.

Goblet cells were identified as periodic acid Schiff-positive cells, and staining was conducted according to the manufacturer's instructions. Briefly, sections were rinsed with distilled water for, oxidised in 0.5% periodic acid solution, followed by washing with distilled water. Slides were then placed in Schiff's reagent, washed in warm running tap water, then counterstained in Gill 3 Haematoxylin.

Statistical analysis

Statistical analyses were performed using GraphPad InStat software (GraphPad Software Inc, La Jolla, CA, USA). Data distributions were determined by the D'Agostino and Pearson normality test. Comparisons between groups were made by an unpaired *t*-test, the Mann–Whitney *U*-test or the Chi-squared test where indicated. Pearson or Spearman's correlations were performed to determine associations between gene expression and other markers of T2 and eosinophilic inflammation.

ACKNOWLEDGMENTS

DS is supported by the National Institute for Health Research (NIHR) Manchester Bio-medical Research Centre (BRC). The authors acknowledge the North West Lung Centre Charity for supporting this project. In addition, we thank the study participants for their contribution. This report is independent research and the views expressed in this publication are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

Data included in this study were obtained from samples collected from a previous bronchoscopy study funded by Medimmune.

CONFLICTS OF INTEREST

DS has received sponsorship to attend and speak at international meetings, honoraria for lecturing or attending advisory boards from the following companies: Aerogen, AstraZeneca, Boehringer Ingelheim, Chiesi, Cipla, CSL Behring, Epiendo, Genentech, GlaxoSmithKline, Glenmark, Gossamerbio, Kinaset, Menarini, Novartis, Pulmatrix, Sanofi, Teva, Theravance and Verona. AH and JD have no conflicts of interest. T-HP and CM are employees of AstraZeneca.

AUTHOR CONTRIBUTIONS

Andrew Higham: Conceptualization; data curation; formal analysis; methodology; writing – original draft; writing – review and editing. **Josiah Dungwa:** Methodology; writing – review and editing. **Christopher McCrae:** Resources; writing – review and editing. **Tuyet-Hang Pham:** Resources; writing – review and editing. **Dave Singh:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; supervision; writing – original draft; writing – review and editing.

REFERENCES

1. Higham A, Quinn AM, Cancado JED, Singh D. The pathology of small airways disease in COPD: historical aspects and future directions. *Respir Res* 2019; **20**: 49.
2. Rutgers SR, Postma DS, ten Hacken NH *et al*. Ongoing airway inflammation in patients with COPD who do not currently smoke. *Thorax* 2000; **55**: 12–18.
3. Rutgers SR, Timens W, Kaufmann HF, van der Mark TW, Koeter GH, Postma DS. Comparison of induced sputum with bronchial wash, bronchoalveolar lavage and bronchial biopsies in COPD. *Eur Respir J* 2000; **15**: 109–115.
4. Higham A, Leow-Dyke S, Jackson N, Singh D. Stability of eosinophilic inflammation in COPD bronchial biopsies. *Eur Respir J* 2020; **56**: 2004167.
5. Kolsum U, Damera G, Pham TH *et al*. Pulmonary inflammation in patients with chronic obstructive pulmonary disease with higher blood eosinophil counts. *J Allergy Clin Immunol* 2017; **140**: 1181–1184.e7.
6. Singh D, Bafadhel M, Brightling CE *et al*. Blood eosinophil counts in clinical trials for chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2020; **202**: 660–671.
7. Singh D, Agusti A, Anzueto A *et al*. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report 2019. *Eur Respir J* 2019; **53**: 1900164.
8. Higham A, Beech A, Wolosianka S *et al*. Type 2 inflammation in eosinophilic chronic obstructive pulmonary disease. *Allergy* 2021; **76**: 1861–1864.
9. George L, Taylor AR, Esteve-Codina A *et al*. Blood eosinophil count and airway epithelial transcriptome relationships in COPD versus asthma. *Allergy* 2020; **75**: 370–380.
10. Andersson CK, Mori M, Bjermer L, Lofdahl CG, Erjefalt JS. Novel site-specific mast cell subpopulations in the human lung. *Thorax* 2009; **64**: 297–305.
11. Bradding P, Arthur G. Mast cells in asthma - state of the art. *Clin Exp Allergy* 2016; **46**: 194–263.
12. Soltani A, Ewe YP, Lim ZS *et al*. Mast cells in COPD airways: relationship to bronchodilator responsiveness and angiogenesis. *Eur Respir J* 2012; **39**: 1361–1367.
13. Gosman MM, Postma DS, Vonk JM *et al*. Association of mast cells with lung function in chronic obstructive pulmonary disease. *Respir Res* 2008; **9**: 64.
14. Andersson CK, Mori M, Bjermer L, Lofdahl CG, Erjefalt JS. Alterations in lung mast cell populations in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010; **181**: 206–217.
15. Winter NA, Qin L, Gibson PG *et al*. Sputum mast cell/basophil gene expression relates to inflammatory and clinical features of severe asthma. *J Allergy Clin Immunol* 2021; **148**: 428–438.
16. Louis RE, Cataldo D, Buckley MG *et al*. Evidence of mast-cell activation in a subset of patients with eosinophilic chronic obstructive pulmonary disease. *Eur Respir J* 2002; **20**: 325–331.
17. Vieira Braga FA, Kar G, Berg M *et al*. A cellular census of human lungs identifies novel cell states in health and in asthma. *Nat Med* 2019; **25**: 1153–1163.
18. Jiang J, Faiz A, Berg M *et al*. Gene signatures from scRNA-seq accurately quantify mast cells in biopsies in asthma. *Clin Exp Allergy* 2020; **50**: 1428–1431.
19. Suurmond J, Habets KLL, Tatum Z *et al*. Repeated FcepsilonRI triggering reveals modified mast cell function related to chronic allergic responses in tissue. *J Allergy Clin Immunol* 2016; **138**: 869–880.
20. Tiotiu A, Badi Y, Kermani NZ *et al*. Association of differential mast cell activation to granulocytic inflammation in severe asthma. *Am J Respir Crit Care Med* 2022; **205**: 397–411.
21. Berry M, Morgan A, Shaw DE *et al*. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax* 2007; **62**: 1043–1049.
22. Gizycki MJ, Hattotuwa KL, Barnes N, Jeffery PK. Effects of fluticasone propionate on inflammatory cells in COPD: an ultrastructural examination of endobronchial biopsy tissue. *Thorax* 2002; **57**: 799–803.
23. Hattotuwa KL, Gizycki MJ, Ansari TW, Jeffery PK, Barnes NC. The effects of inhaled fluticasone on airway inflammation in chronic obstructive pulmonary disease: a double-blind, placebo-controlled biopsy study. *Am J Respir Crit Care Med* 2002; **165**: 1592–1596.
24. James A, Gyllfors P, Henriksson E *et al*. Corticosteroid treatment selectively decreases mast cells in the smooth muscle and epithelium of asthmatic bronchi. *Allergy* 2012; **67**: 958–961.
25. Dougherty RH, Sidhu SS, Raman K *et al*. Accumulation of intraepithelial mast cells with a unique protease phenotype in T_H2-high asthma. *J Allergy Clin Immunol* 2010; **125**: 1046–1053.e8.
26. Wang G, Baines KJ, Fu JJ *et al*. Sputum mast cell subtypes relate to eosinophilia and corticosteroid response in asthma. *Eur Respir J* 2016; **47**: 1123–1133.

© 2022 The Authors. *Clinical & Translational Immunology* published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

27. Weidner N, Austen KF. Heterogeneity of mast cells at multiple body sites. Fluorescent determination of avidin binding and immunofluorescent determination of chymase, tryptase, and carboxypeptidase content. *Pathol Res Pract* 1993; **189**: 156–162.
28. KleinJan A, Godthelp T, Blom HM, Fokkens WJ. Fixation with Carnoy's fluid reduces the number of chymase-positive mast cells: not all chymase-positive mast cells are also positive for tryptase. *Allergy* 1996; **51**: 614–620.
29. Yousuf A, Ibrahim W, Greening NJ, Brightling CE. T2 Biologics for Chronic Obstructive Pulmonary Disease. *J Allergy Clin Immunol Pract* 2019; **7**: 1405–1416.
30. Singh D, Agusti A, Martinez FJ *et al*. Blood eosinophils and chronic obstructive pulmonary disease: a global initiative for Chronic Obstructive Lung Disease Science Committee 2022 review. *Am J Respir Crit Care Med* 2022; **206**: 17–24.
31. Higham A, Karur P, Jackson N, Cunoosamy DM, Jansson P, Singh D. Differential anti-inflammatory effects of budesonide and a p38 MAPK inhibitor AZD7624 on COPD pulmonary cells. *Int J Chron Obstruct Pulmon Dis* 2018; **13**: 1279–1288.
32. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014; **15**: 550.
33. Bhakta NR, Solberg OD, Nguyen CP *et al*. A qPCR-based metric of Th2 airway inflammation in asthma. *Clin Transl Allergy* 2013; **3**: 24.
34. Southworth T, Van Geest M, Singh D. Type-2 airway inflammation in mild asthma patients with high blood eosinophils and high fractional exhaled nitric oxide. *Clin Transl Sci* 2021; **14**: 1259–1264.
35. Chtanova T, Newton R, Liu SM *et al*. Identification of T cell-restricted genes, and signatures for different T cell responses, using a comprehensive collection of microarray datasets. *J Immunol* 2005; **175**: 7837–7847.
36. Jayapal M, Tay HK, Reghunathan R *et al*. Genome-wide gene expression profiling of human mast cells stimulated by IgE or FcεRI-aggregation reveals a complex network of genes involved in inflammatory responses. *BMC Genomics* 2006; **7**: 210.
37. Nagarkar DR, Ramirez-Carrozzi V, Choy DF *et al*. IL-13 mediates IL-33-dependent mast cell and type 2 innate lymphoid cell effects on bronchial epithelial cells. *J Allergy Clin Immunol* 2015; **136**: 202–205.
38. Okumura S, Kashiwakura J, Tomita H *et al*. Identification of specific gene expression profiles in human mast cells mediated by Toll-like receptor 4 and FcepsilonRI. *Blood* 2003; **102**: 2547–2554.

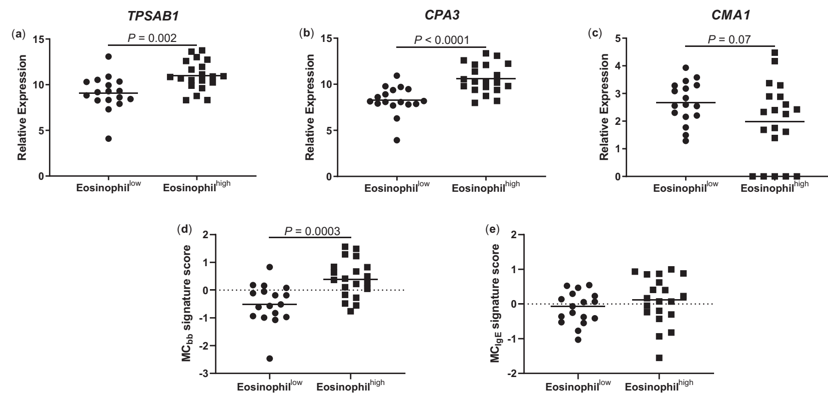
Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main.



Our results demonstrate that eosinophilic inflammation is associated with altered mast cell characteristics in chronic obstructive pulmonary disease (COPD) patients, implicating mast cells as a component of T2 inflammation present in a subset of COPD patients.