Benralizumab's anti-eosinophil efficacy may be decreased by impaired NK cell activity

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To the Editor:

We read with interest the report by Dagher *et al.* [1] of a novel anti-eosinophil action of benralizumab. Benralizumab was shown to induce eosinophil apoptosis by a macrophage-derived tumour necrosis factor (TNF)-induced caspase 3/7 activation, and this was amplified by interferon-γ (IFN-γ) secreted from natural killer (NK) cells. Indeed, in patients with severe asthma, including those who are dependent on daily systemic glucocorticosteroids for asthma control, benralizumab effectively depletes sputum eosinophils [2]. Although no direct head-to-head comparisons have been made, eosinophil suppression is likely to be greater than with the currently approved dose of mepolizumab [3], leading to greater clinical efficacy [4]. However, there may be instances when the anti-eosinophil activity of benralizumab may be impaired due to decreased NK cell numbers or activity that are described in patients with severe asthma on high doses of glucocorticosteroids [5].

A 34-year-old female with severe airway hyperresponsiveness and mild sinus and asthma symptoms was seen in 2012, shortly after she had a baby, with dyspnoea, diffuse alveolar haemorrhage, left ventricular ejection fraction of 25%, skin vasculitis and peak blood eosinophils of 13.9 cells·μL^{-1}. Antineutrophil cytoplasmic antibodies (ANCA) were not detected in serum. However, it was detected in sputum in low titres, along with other autoantibodies such as anti-MARCO. She was treated with high doses of systemic corticosteroids and intravenous cyclophosphamide (10 doses), and later switched to oral prednisone and azathioprine. She participated in a clinical trial of subcutaneous mepolizumab 300 mg monthly for eosinophilic granulomatosis with polyangiitis (EGPA) [6], received the active drug, and successfully tapered her daily prednisone from 35 mg to 5 mg, and her asthma remained well controlled on additional high doses of inhaled corticosteroids and long-acting beta-agonists. Her sputum and blood eosinophil levels were 0 after 4 years of follow-up. Mepolizumab had to be discontinued in 2018 when she finished participating in the open-label extension of the clinical trial, and the drug was not re-imbursed by the provincial insurance plan. Her vasculitis did not flare but her asthma exacerbated, with a 1-L decrease in forced expiratory volume in 1 s (FEV_1), and her blood eosinophils rose to 0.5 cells·μL^{-1}. Prednisone dose had to be increased to 15 mg daily, which suppressed eosinophils to 0.1 cells·μL^{-1}. Given her significant adverse reactions to prednisone, including suicidal psychosis, she was then switched to benralizumab to attempt steroid weaning. She received 30 mg subcutaneously monthly for 3 months, and her prednisone was reduced by 2.5 mg daily after each injection. She noticed increasing shortness of breath with prednisone reduction and, at 7.5 mg daily prednisone, had an exacerbation with a reduction in FEV_1 to 2.24 L (77% predicted) and increase in sputum eosinophils to 78% and blood eosinophils to 0.4 cells·μL^{-1}. She did not have a relapse of any other EGPA-manifestations (Birmingham Vasculitis Activity Score score 1). We re-examined sputum after her fourth benralizumab injection and it showed 58% eosinophils, along with many free granules and Charcot Leyden crystals.

We examined peripheral blood NK cell numbers and function (1 week after the fourth benralizumab injection), as previously described [7, 8], as a possible reason for lack of efficacy of benralizumab. NK cell numbers (11% of total circulating $CD45^+$ cells) were within normal range (figure 1a and b). CD16 (the receptor that recognises the Fc portion of benralizumab) expression was also normal (96.5%), as was KIR (killer-cell immunoglobulin-like receptor) expression. However, NK cell function of antibody-dependent cytotoxicity (ADCC) in the patient, as assessed by a tumour killing assay to trastuzumab (another drug that

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Although likely to be exceedingly rare, impaired ADCC due to NK cell dysfunction needs to be considered as one of the reasons, along with the development of anti-drug neutralising antibodies, for impaired anti-eosinophil activity of benralizumab https://bit.ly/3nxeFVg

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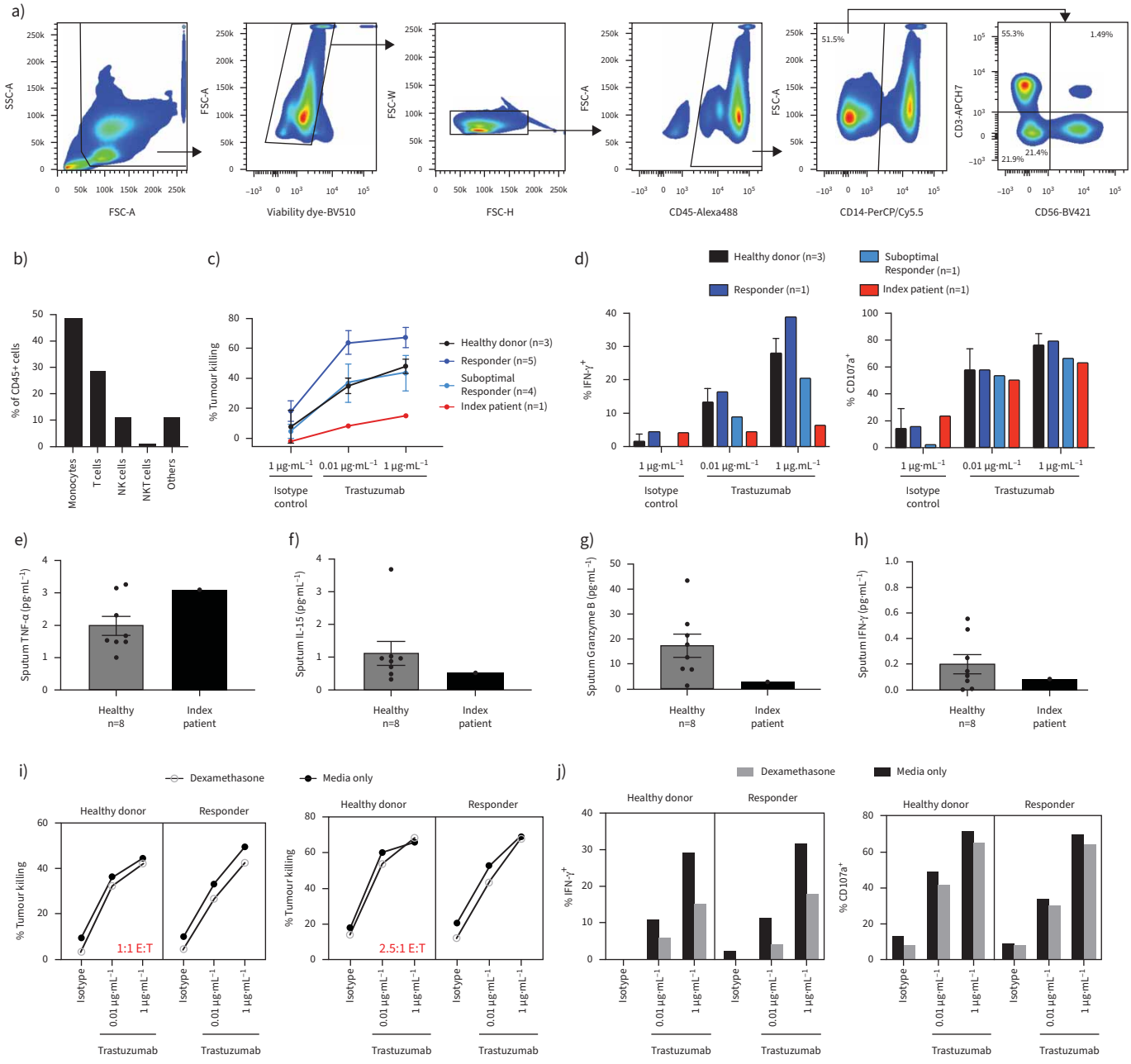

FIGURE 1 Suboptimal response to benralizumab corresponds with impaired natural killer (NK) cell antibody-mediated functions. a, b) Peripheral blood mononuclear cells were isolated from the patient's peripheral blood, seeded at 1×10^6 cells per well and stained with extracellular markers for immunophenotyping *via* flow cytometry. a) Gating strategy on immune cell populations *via* CD14, CD3 and CD56 expression. b) Proportion of monocytes ($CD14^+$), T cells ($CD14^-CD56^-CD3^+$), NK cells ($CD14^-CD56^+CD3^-$), NK T cells ($CD14^-CD56^+CD3^+$) and other (CD14–CD56–CD3–) immune cell subsets of $CD45^+$ cells. c) NK cells were isolated from the peripheral blood of healthy donors (n=3), severe asthmatics with good (n=5) or suboptimal (n=4) response to benralizumab, and the index patient plotted separately (n=1, red). d) NK cells were seeded at 200 000 cells per well and incubated with $HER2^+$ SKBR3 breast cancer target cells with the indicated concentrations of trastuzumab or the corresponding isotype control. Percent tumour killing and NK cell interferon-γ (IFN-γ) and CD107a expression was assessed after 5 h of incubation *via* flow cytometry. Percent tumour killing was assessed for n=1 of optimal, suboptimal benralizumab responder, along with index patient, and n=3 healthy donors. Results are represented as mean±SD of three technical repeats for healthy bars. NK cell activity-associated cytokine profile in cell-free sputum supernatant of index patient plotted for e) tumour necrosis factor (TNF)-α, f) interleukin (IL)-15, g) granzyme B and h) IFN-γ, compared to sputa from eight healthy subjects. Cytokines were assessed on Ella automated ELISA. Finally, NK cells were seeded at 200 000 cells per well and incubated at a 1:1 or 2.5:1 target to effector cells ratio with $HER2^+$ SKBR3 breast cancer target cells with the indicated concentrations of trastuzumab or the corresponding isotype control. Percent tumour killing in presence/absence of 10^{-5} M dexamethasone (i) and IFN-γ and CD107a expression in NK cells (j) were assessed after 5 h of incubation *via* flow cytometry. Experimental means from n=3 triplicate wells are plotted for each condition.

exerts its effect by NK-cell-mediated ADCC) was impaired compared to NK cells from healthy donors (n=3) and in relation to patients with severe prednisone-dependent asthma (n=5) who had good response to benralizumab (figure 1c) [7]. Activation of NK cells as evidenced by release of IFN-γ and LAMP-1 ($CD107a^+$) was also impaired in response to trastuzumab compared to healthy (n=3) donors (figure 1d). In the airway, TNF-α levels in sputum supernatant were comparable to those in eight healthy controls, but IL-15, and markers of NK cell activation (granzyme B and IFN-γ), were reduced, consistent with impaired ADCC (figure 1e–h). The proportion of $CD14^+$ cells among the CD45 cells were normal (48.5%), with 19% of them expressing CD16 and, therefore, unlikely to be contributing to impaired response to benralizumab. Amongst other $CD45^+$ cells, T cells were ∼28% and NK T cells are virtually absent (<1%) (figure 1b). To further evaluate the role of corticosteroids on NK cell activity, we performed *in vitro* experiments, where NK cells from a healthy donor and benralizumab responder were incubated with and without dexamethasone (10^{-5} M). The ADCC (figure 1i) and CD107a expression were comparable but there was a two-fold reduction in IFN-γ expression with dexamethasone (figure 1j). All measurements conducted on samples from the patient were done with the patient's consent and with the approval of the local research ethics board for NK cell function.

Benralizumab was therefore discontinued. We obtained mepolizumab through a compassionate programme, and after 3 monthly injections of 300 mg, blood and sputum eosinophils are zero, FEV_1 has improved to 2.58 L (85% pred), prednisone has been reduced to 2.5 mg daily, and asthma control is excellent (Asthma Control Questionnaire score 0). In our patient, NK cell dysfunction may be due to a number of factors, including previous corticosteroid use or disease severity, which likely occurred prior to initiating benralizumab. Indeed, Duvall *et al.* [5] demonstrated that severe asthmatics have impaired NK cell cytotoxicity, where corticosteroids can further impair NK cell function. Moreover, we showed that dexamethasone may reduce IFN-γ expression, which can further impair eosinophil apoptosis. Additionally, azathioprine may also lead to NK cell deficiency [9]; however, cyclophosphamide likely is a non-contributor. In fact, cyclophosphamide is reported to enhance NK cell activity [10]. Finally, we suspect the patient's suboptimal response to benralizumab may be partly due to *de novo* development of anti-drug antibodies to the biologic, given her inherent predisposition to autoimmunity and aberrant immune response (EGPA is a systemic autoimmune disease and our index patient has evidence of sputum ANCA). Indeed, there is growing evidence of such immune irregularity on biologic therapies [11].

In summary, we present a clinical scenario of a patient with EGPA who had suboptimal response to benralizumab (as demonstrated by lack of eosinophil depletion), which could be due to impaired NK-cell activity. Given the importance of NK cell-released IFN-γ in macrophage-mediated eosinophil apoptosis [1], our findings suggest that dysfunction in both NK cell-mediated ADCC and antibody-induced IFN-γ may contribute to this suboptimal response. Although likely to be exceedingly rare, this needs to be considered as one of the reasons, along with the development of anti-drug neutralising antibodies, for impaired anti-eosinophil activity of benralizumab.

Sophie M. Poznanski[1], Ana Portillo[1], Manali Mukherjee[2], Anurag Bhalla[2], Kiho Son[2], Ali A. Ashkar[1], Nader Khalidi[3] and Parameswaran Nair[2]

[1]Division of Clinical Immunology and Allergy, McMaster University and St Joseph's Healthcare, Hamilton, ON, Canada. [2]Division of Respirology, McMaster University and St Joseph's Healthcare, Hamilton, ON, Canada. [3]Division of Rheumatology, McMaster University and St Joseph's Healthcare, Hamilton, ON, Canada.

Corresponding author: Parameswaran Nair (parames@mcmaster.ca)

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References

1 Dagher R, Kumar V, Copenhaver AM, *et al.* Novel mechanisms of action contributing to benralizumab's potent anti-eosinophilic activity. *Eur Respir J* 2022; 59: 2004306.

2 Sehmi R, Lim HF, Mukherjee M, *et al.* Benralizumab attenuates airway eosinophilia in prednisone-dependent asthma. *J Allergy Clin Immunol* 2018; 141: 1529–1532.e1528.

3 Moran AM, Ramakrishnan S, Borg CA, *et al.* Blood eosinophil depletion with mepolizumab, benralizumab, and prednisolone in eosinophilic asthma. *Am J Respir Crit Care Med* 2020; 202: 1314–1316.

4 Kavanagh JE, Hearn AP, d'Ancona G, *et al.* Benralizumab after sub-optimal response to mepolizumab in severe eosinophilic asthma. *Allergy* 2021; 76: 1890–1893.

5 Duvall MG, Barnig C, Cernadas M, *et al.* Natural killer cell-mediated inflammation resolution is disabled in severe asthma. *Sci Immunol* 2017; 2: eaam5446.

6 Wechsler ME, Akuthota P, Jayne D, *et al.* Mepolizumab or placebo for eosinophilic granulomatosis with polyangiitis. *N Engl J Med* 2017; 376: 1921–1932.

7 Poznanski SM, Mukherjee M, Zhao N, *et al.* Asthma exacerbations on benralizumab are largely non-eosinophilic. *Allergy* 2021; 76: 375–379.

8 Poznanski SM, Ritchie TM, Fan IY, *et al.* Expanded human NK cells from lung cancer patients sensitize patients' PDL1-negative tumors to PD1-blockade therapy. *J Immunother Cancer* 2021; 9: e001933.

9 Orandi AB, Vogel TP, Keppel MP, *et al.* Azathioprine-associated complete NK cell deficiency. *J Clin Immunol* 2017; 37: 514–516.

10 Sharma B, Vaziri ND. Augmentation of human natural killer cell activity by cyclophosphamide *in vitro*. *Cancer Res* 1984; 44: 3258–3261.

11 Pérez-De-Lis M, Retamozo S, Flores-Chávez A, *et al.* Autoimmune diseases induced by biological agents. A review of 12,731 cases (BIOGEAS Registry). *Expert Opin Drug Saf* 2017; 16: 1255-1271.