The damaging and protective features of eosinophils in healthy individuals and patients with chronic inflammatory respiratory diseases

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Chapter 8

General discussion & Summary

GENERAL DISCUSSION

Together the *in vitro* studies in **chapter 2 and 3** showed that human eosinophils rapidly bind and inactivate virus and thus may therefore be important scavengers of virus in the mucosa. Therefore, eosinophils can prevent further viral propagation. The biological significance of virus inactivation by eosinophils was shown both in a murine model and in the human study. With the IL-5 transgenic mice we found a more rapid clearance of influenza and a lower morbidity compared to controls. Earlier murine studies showed that the number of eosinophils influenced pneumonia virus of mice (PVM) mortality, although the anti-viral mechanisms proposed were distinct from those shown in our study (1-4). In our human mild asthma patient study we depleted eosinophils using anti-IL-5 (Mepolizumab) followed by a RV16 challenge. This resulted in an enhanced virus titer in asthma patients depleted from eosinophils compared to placebo-treated asthma patients. In line with these results we showed that eosinophils from asthma patients display a reduced capacity to internalize and inactivate virus, possibly because eosinophils have already been activated in the circulation. Thus, eosinophil depletion in asthma patients is expected to lower the viral load less compared to that by depletion of eosinophils with a normal anti-viral capacity. Together these findings underline the anti-viral activity of eosinophils.

The mechanism by which eosinophils reduce the infectivity of viruses is as yet unknown. The major eosinophil granular constituents are eosinophil cytotoxic protein (ECP) and eosinophil- derived neurotoxin (EDN) exert anti-viral activities and ribonuclease activity (5). Our results in **chapter 2** demonstrate that viral particles are internalized and may end up in the lysosomal compartment. By electron microscopy we found intact viral particles in the cytoplasm, but we failed to find viral particles enclosed by a membrane, which may indicate that viral particles are rapidly degraded. For the infectious cycle, RSV fuses with the plasma membrane (6, 7) and so the presence of intact viral particles is indicative of the uptake of virus by eosinophils. Both the number of eosinophils and their activation state control their antiviral response, but the precise mechanisms by which viruses are inactivated by eosinophils and how viruses are bound are still unknown.
In **chapter 3**, we showed that Mepolizumab, an anti-IL-5-Ab, potently reduce eosinophils in peripheral blood and in sputum, but not in bronchoalveolar lavage fluid (BALF) in mild asthmatic patients, as was also shown in other studies (8, 9). Furthermore, we showed that Mepolizumab affected NK cells and B-cells in blood and CD8-T cells and B-cells in BALF. We additionally showed that Mepolizumab blocked the RV16-induced increase of neutrophils in sputum and MPO, a neutrophil activation marker, in BALF. Indicating both innate as adaptive immune responses are affected by Mepolizumab. In

line with these findings, others showed in a murine study that eosinophils enhanced viral immunity by enhancing CD8 T cell responses against the influenza virus in an antigen specific manner (10). These findings also lead to a new perspective of therapeutic benefits in reducing eosinophils in the airway. Perhaps a lower dose of anti-IL-5 should be aimed at to prevent eosinophil activation, as discussed in *chapter 2 and 5*, rather than to reduce eosinophil numbers. Furthermore, in line with other studies, we showed that lung function and clinical asthma symptoms are not improved by the treatment of mild asthma patients (11-13). In fact, attenuation of eosinophils without affecting asthma pathophysiology allowed us to conclude that the observed effects in the study were related to a direct effect of mepolizumab and not indirectly by modifying asthma pathophysiology.

Interestingly, we did not find dramatic differences between the groups stratified in </≥3% eosinophils, whereas an increased effect of Mepolizumab in ≥3% group was expected, simply as more eosinophils were present. In studies investigating the effect of Mepolizumab in severe eosinophilic asthma patients, a marked effect on FEV1 and on ACQ, with a reduced number of exacerbations were found (8). This indicates that in mild asthma eosinophils do drive both innate and adaptive immune responses, but may not or to a limited extent (during viral infection) contribute to asthma pathophysiology.
In line with these findings we showed in **chapter 4** that eosinophils are not activated to produce ROS in stable mild asthma, which could explain why Mepolizumab does not improve clinical symptoms as we described *in chapter 3.* On the other hand, MDA levels were significantly increased in the placebo group after RV16 challenge when looking into ≥3% group. RV16 challenge also resulted in a significant increase in nitrotyrosine in the placebo group. Nevertheless, this was lost after stratifying in <3% and ≥3% eosinophils, suggesting that nitrotyrosine production was not directly related to eosinophil counts. This could indicate that, upon virus-induced loss of asthma control, eosinophils contribute to oxidative stress.

A potential alternative approach that may differentially affect protective and damaging properties of eosinophils is the use of inhibitors of phosphodiesterase-4 (PDE4), which is prominently expressed in eosinophils (14). Our findings in **chapter 5** showed that eosinophil activation and specifically degranulation of ECP at baseline differs between asthmatic and healthy subjects. In addition, eosinophils from patients showed a reduced Nox2 activity. The limited Nox2 response to viral exposure by eosinophils from patients is unlikely to be due to a defective Nox2, as the fMLP- induced Nox2 activity is significantly higher. This points to a defective response to virus by eosinophils from patients, in line with our earlier observation in *chapters 2 and 3*, that eosinophils from

asthma patients have a reduced capacity to capture and inactivate virus. The inhibitory effect of PDE4 inhibitors was more profound on eosinophils from healthy controls compared to those from asthma patients. At baseline and after stimulation by fMLP and influenza, the two PDE4 inhibitors reduced the Nox2 activity significantly. Different studies showed an effect of PDE4 inhibitors on the number of eosinophils (15, 16), but not specifically on eosinophil inflammatory markers. However, in line with our findings on Nox2 inhibition, it was shown that PDE4 inhibitors can reduce superoxide production in both eosinophils as neutrophils (17). Showing that PDE4 inhibitors can enhance the virus-induced response by eosinophils and in particular attenuate the Nox2 activity by eosinophils from asthmatics, this underlines a potential role for PDE4 in controlling virus-induced responses that could be relevant in asthma and its exacerbations.

In chapter 6, we studied the effect of azithromycin (AZM) in bronchiectasis patients with the co- diagnosis of asthma and COPD in relation to their airway inflammatory profile. Altenburg et al. (18) showed that the frequency of exacerbations was significantly different in patients treated with AZM compared to placebo control. Also, lung function, disease symptoms and quality of life improved in the AZM arm compared to placebo control. We found that at baseline the airway inflammatory profile was comparable in the total population, asthma patients, COPD patients and “other” patients. This indicates that the inflammatory profile in patients with bronchiectasis bears no relation to the co-diagnosis. The number of exacerbations in the past year correlated to IL-21, MMP9, MPO and TNF-α in the underlying pathologies asthma and IP-10/CXCL10 the other group. These parameters could therefore be used to predict exacerbations in patients. Unfortunately, because of a limited power and low number of patients in the COPD group we could not state that AZM treatment alters the inflammatory profile. This is in contrast to AZM studies, which indicated that macrolide antibiotics are not only bactericidal but also anti-inflammatory and are therefore favored in treatment (19-21).
Relative sputum eosinophil counts by cell differentiation are the gold standard for phenotyping and treating adult patients with asthma. The procedure for sputum cell differentiation, however, is a relative lengthy and labour-intensive procedure and requires trained personnel. We examined in **chapter 7** whether it is possible to replace this technique by spectral analysis. Eosinophils contain granules in which various unique constituents are stored, one of which is eosinophil peroxidase (EPO), neutrophils on the other hand contain neutrophilic myeloperoxidase (MPO). Both EPO and MPO are abundantly present in eosinophils and neutrophils respectively, and both are heme- containing enzymes enabling specific detection by spectroscopy (22). Although the spectroscopic method is accurate as it assesses all cells, the amount of MPO may be slightly underestimated by about 2-3% as the isosbestic point of EPO and

the peak for MPO in the redox spectrum are slightly off. The amount of sputum received is unfortunately a limiting factor, nevertheless this could be a good method for high sputum counts in diseases like COPD and bronchiectasis. This approach could also be used to determine eosinophils and neutrophils in bronchoalveolar lavage, however, this await further experiments.

CONCLUSIONS

1. Respiratory syncytial virus and Influenza are internalized and inactivated by eosinophils.
2. Eosinophils' capacity to capture virus is reduced with increasing asthma severity.
3. Mepolizumab reduced both eosinophil activation and numbers, but did not prevent activation of the remaining eosinophils in response to a rhinovirus 16 challenge in mild asthma patients.
4. RV16 load is increased in Mepolizumab-treated mild asthma patients.
5. Eosinophils do contribute to both oxidative and nitrosative stress after RV16-induced asthma exacerbations, but not in stable asthma.
6. PDE4 inhibitors can negatively modulate eosinophil activation, but does not affect virus binding.
7. The inflammatory profile in bronchiectasis is similar between various etiologies, this indicates that the underlying mechanisms of inflammation is different.
8. We showed no evidence for an anti-inflammatory effect of Azithromycin in bronchiectasis.

FUTURE PERSPECTIVE

In this thesis I discussed the role and function of eosinophils during innate immune responses to virus. Eosinophils are not only able to initiate an anti-viral response, they can also drive both innate and adaptive immune responses. Considering current treatments focusing on depletion of eosinophils in asthma patients, caution is necessary since not only eosinophils are hereby affected. This could potentially lead towards a defective immune response, necessary to counter both various pathogens (parasite, yeast) as well as tumor formation.

Treatment should therefore be more focused upon reducing specific eosinophil activity instead of eliminating eosinophil numbers. It would be very interesting to examine the underlying pathogenesis of eosinophils during asthma exacerbations. When the mechanism behind exacerbations are known, it becomes easier to understand in which processes we should intervene. For instance, it was shown that asthma exacerbations have been associated with an increase in sputum eosinophils. It would be interesting to examine the effects of both eosinophil numbers, activation, degranulation and cell trap formation in these patients; before, during and after an asthma exacerbation.

The mechanism by which eosinophils take up viruses and reduce their infectivity is as yet unknown, therefore it is interesting to further investigate these mechanisms. It is unlikely that viruses were bound by expelled DNA as occurs in eosinophils extracellular trap cell death (EETosis), since in our samples all nuclei and cells were intact (23). More likely, granular eosinophilic compounds like MBP facilitate virus binding, which was found on the eosinophil's cell surface. It was already demonstrated by others that MBP binds to the cell surface via heparan sulfate proteoglycan (24). Influenza and RSV co-localized with MBP on the eosinophil cell surface (unpublished data), suggesting that an electrostatic interaction between the negatively charged virus and the positively charged MBP might facilitate viral binding. After the initial binding, viral particles clustered but separately from MBP, similar to the well-recognized capping phenomenon (25), which suggests that there is active sorting at the cellular membrane after which the viral particles are taken up. The eosinophilic compound that mediates viral capture may prevent binding of the virus to target cells, thereby reducing infectivity. It is possible that other granule proteins exert a similar function. The major granular constituents ECP and eosinophil-derived neurotoxin (EDN) exert anti-viral and ribonuclease activity (5). Although this working mechanism has been proposed for extracellular action, after internalization, the RNAse-activities of ECP and EDN may also contribute to inactivate viruses. Our data (unpublished) unveil that viral particles are indeed internalized and end up in the lysosomal compartment. Interestingly, in recent studies (26-28) evidence is provided that RSV and influenza A enter the lysosomal compartment in various cell types, which either leads to viral replication or its abortion. There are no previous observations of viral uptake in lysosomes by eosinophils and our finding, that shows it reduces infectious RSV, suggests that the abortive pathway is involved. However, future research is necessary to validate the mechanism.

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SUMMARY

Respiratory virus infections in allergic asthmatics promote accumulation of eosinophils and aggravate allergic inflammation in airways. Recent studies demonstrated that eosinophilic granular constituents exert antiviral activity, suggestive of protective properties by eosinophils. However, little is known about the actual role of eosinophils in immune modulation and in virus- induced exacerbations in respiratory diseases.

In **chapter 2**, we showed that eosinophils are capable of rapid capture and inactivation of virus, which has been so far an unrecognized property of eosinophils. In addition, we found that eosinophils particularly from severe asthma patients are defective in binding and, by that probably also in antiviral activity, which may lead to enhanced viral loads. These reduced anti-viral and enhanced activation of eosinophils from asthma patients likely underlie, at least in part, the pathogenesis of virus-induced asthma exacerbations.

In **chapter 3**, we showed that Mepolizumab, an anti-IL-5 therapeutic aimed at reducing eosinophils *in vivo*, not only reduced eosinophil number but also that of lymphocytes. Although Mepolizumab attenuated eosinophil numbers and activation, it did not affect lung function and FeNO. Mepolizumab enhanced circulating NK cells at baseline and upon RV16 challenge it prevented reduction of B lymphocytes and macrophages in BALF and an increase of neutrophils and their activation. These findings lead to a new perspective of therapeutic benefits in reducing eosinophils in the airway. Novel therapeutics like anti-IL-5 and IL-5R are currently aimed at reducing eosinophils and eosinophil cytotoxic compounds, however, consequently also reduce the potential protective and immune-modulatory properties of eosinophils. Based on the apparent maintenance of the anti-viral properties of eosinophils from mild to moderate asthma patients by corticosteroids, we speculated that tapering anti-IL-5 and IL-5R treatments to limit activation of eosinophils, rather than eradicate eosinophils, may lead to even better clinical results.

In **chapter 4**, we showed by the analysis of different oxidative stress markers that eosinophils in the same RV16-challenged mild to moderate asthma patients treated with Mepolizumab do contribute to ROS formation as reflected by increased levels of malondialdehyde (MDA) and nitrotyrosine. However, this is not seen during stable condition in those mild to moderate asthma patients. Therefore, we believe that besides the previously mentioned cytotoxic properties, eosinophils could also contribute to the pathogenesis of asthma due to local oxidative stress induced by RV16.

In **chapter 5**, we described a study in which we explored the effect of two PDE4 inhibitors (CHF6001, CHD22880) on peripheral blood eosinophils and their functions, particularly in relation to activation of eosinophils by respiratory viruses. We analyzed eosinophils from healthy controls and patients with mild or severe asthma and, in comparison, neutrophils. Eosinophils, but not neutrophils, from asthmatics are different in activation (CD69) and degranulation (ECP) at baseline compared to healthy controls. Eosinophils in response to virus display a pronounced NADPH oxidase (Nox2) activity, but this is reduced in those from asthma patients. Interestingly this effect of PDE4 inhibitors is specific for eosinophils, since neutrophils do not show this response. Although PDE4 inhibitors do not significantly affect activation of neither healthy eosinophils nor neutrophils, PDE4 inhibitors do markedly inhibit Nox2 activity in eosinophils from patients. Interestingly, eosinophils from severe asthma patients showed a reduced virus binding as compared to eosinophils from either healthy or mild asthmatics and PDE4 inhibitors had no effect on virus binding by eosinophils.

In **chapter 6**, we examined the inflammatory profile in patients suffering from either treated or untreated bronchiectasis. The BAT study (Bronchiectasis and long-term Azithromycin Treatment) was a multicenter, double-blind, placebo-controlled study in 83 non-CF bronchiectasis patients. In total 43 patients received Azithromycin (AZM) therapy and 40 placebo. Daily use of AZM for 12 months resulted in a reduced rate of infectious exacerbations compared to control. Sputum was collected each 3 months and analyzed for inflammatory mediators and eosinophil activation and degranulation. At baseline, both patient characteristics and inflammatory profile are similar in the total population. The inflammatory profiles when split based upon the co-diagnosis asthma, COPD and others were also similar. This indicates that the typical asthma and COPD inflammatory profile is not detected in bronchiectasis patients and therefore bronchiectasis determine the inflammatory profile instead of the co-diagnosis. When the number of exacerbations of the past year were correlated to the inflammatory profile, in the total group we showed a correlation of ECP, IP-10, IL-8, MMP9, MPO and VEGF. Interestingly, in asthma IL-21, MMP9, MPO and TNF-α correlated to the number of exacerbations while in COPD no mediators correlated. Surprisingly, AZM in time did not affect inflammation in asthma, COPD or in the “other” group, however, this should be verified in a larger study.

In **chapter 7**, we showed a simple spectral technique that can substitute for relative eosinophil and neutrophil sputum counts. Since the gold standard technique of relative cell count is labour intensive, time consuming and requires specific expertise, we developed a quick and easy 15- minute test. Whole induced sputum samples were used, which could contain more squamous

epithelial cells than for example selected plugs. Interestingly, even sputa contaminated with >80% of squamous cells yielded EPO/MPO values that correlated well with cell differentiation data. This combining with the fact that the relative eosinophil and neutrophil counts but not the EPO/MPO ratios are affected by the presence of other cells, this technique evaluating these ratios likely provides a more accurate reflection of eosinophilic and neutrophilic inflammation.

NEDERLANDSE SAMENVATTING

Inleiding

Astma is een chronische ontstekingsziekte van de luchtwegen, waaraan wereldwijd ongeveer 300 miljoen mensen lijden. De oorzaak van astma is een samenspel van genetische en omgevingsfactoren die per patiënt variëren waardoor de wijze waarop astma zich manifesteert kan verschillen tussen patiënten. Frequente virus infecties tijdens de eerste levensjaren kunnen resulteren in de ontwikkeling van astma. Eenmaal behept met astma, dan kunnen deze virus infecties astma verergeren. Omgevingsfactoren zoals allergenen, luchtverontreiniging kunnen net als virus infecties ook een astma aanval veroorzaken, die ook wel exacerbatie wordt genoemd. Tijdens zo een exacerbatie komen er veel afweercellen in de luchtwegen terecht. Deze toegenomen ontstekingsprocessen zorgen voor een slechtere longfunctie, verminderen de kwaliteit van leven en kunnen zelfs levensbedreigend zijn in ernstige astmapatiënten.

Normaal gesproken is een ontstekingsreactie een korte en belangrijke reactie op beschadiging of infecties. In astma is deze reactie chronisch en zorgt daardoor voor schade aan de luchtwegen. Een belangrijk cel type betrokken bij deze reactie in astmapatiënten zijn eosinofiele granulocyten.

In **hoofdstuk 2** wordt beschreven dat eosinofiele granulocyten niet alleen in staat zijn om luchtweg virussen te binden, maar deze ook kapot kunnen maken zodat de virussen niet goed meer een infectie kunnen veroorzaken. In een muizen studie, waarin gebruik wordt gemaakt van een allergisch astma model, laten we zien dat eosinofielen van belang zijn in het verminderen van een virus infectie. Muizen die veel eosinofielen hebben, hadden niet alleen een betere longfunctie maar ook een minder ernstige infectie. Dit werd verder ook aangetoond in mensen met astma die een verlaagd aantal eosinofielen hadden door middel van een medicijn dat eosinofielen verlaagd. Deze groep patiënten heeft meer virus in vergelijking tot astmapatiënten met een normaal aantal eosinofielen. Daarentegen zien we wel dat de groep met hele actieve eosinofiele (CD69) ook een slechtere longfunctie hebben (FEV1). Tot slot werd ook duidelijk dat eosinofiele granulocyten van gezonde personen beter virus binden dan die van patiënten met mild/matig astma en nog beter dan die van patiënten met ernstig astma. Blijkbaar is er een balans nodig tussen gezonde functionele niet te actieve eosinofielen die het virus kunnen binden en op kunnen ruimen.

In **hoofdstuk 3** wordt een patiënten studie beschreven waarin het effect van anti-IL5 (Mepolizumab) wordt geanalyseerd. Dit medicijn zorgt ervoor dat het aantal

eosinofielen in het bloed afneemt. In deze studie van 19 mild tot matige astmapatiënten hebben 8 patiënten een medicijn gekregen, de overige 11 patiënten hebben een placebo behandeling gehad. Behandeling met Mepolizumab zorgt er niet alleen voor dat eosinofielen in aantal en qua activatie verminderen, maar beïnvloedt ook andere cellen en stoffen die van belang zijn voor een beschermende afweerreactie. Daarbij komt ook dat de patiënten die de Mepolizumab behandeling hebben gekregen meer virus infectie hebben in vergelijking met de placebo behandeling. Is behandeling met Mepolizumab in astma dan wel zo'n goed idee?

In **hoofdstuk 4** wordt een subanalyse naar merkers van oxidatieve schade beschreven in materiaal afkomstig van patiënten beschreven in hoofdstuk 3, omdat we verwachten dat eosinofielen granulocyten deze oxidatieve schade bevorderen. Wat hier duidelijk naar voren kwam, is dat tijdens stabiel astma eosinofiele granulocyten niet verantwoordelijk zijn voor oxidatieve schade. Tijdens verergering van astma door de blootstelling aan het verkoudheidsvirus zien we duidelijk dat twee merkers van oxidatieve schade sterk omhooggaan in de placebo groep. Waarvan duidelijk een specifieke indicator (MDA), ook wanneer er alleen gekeken wordt bij patiënten behandeld met placebo met extra veel eosinofiele granulocyten. Dit betekent dat eosinofiele granulocyten pas een bijdrage leveren aan oxidatieve stress wanneer er geen astma controle meer is.

In **hoofdstuk 5** wordt beschreven dat een alternatieve astmatherapie, met zogenaamde PDE4 remmers, goed in staat is om de activatie van zowel eosinofiele als neutrofiele granulocyten te verminderen. In deze studie werd ook duidelijk dat niet alleen beide type granulocyten van gezonde vrijwilligers anders zijn dan die van astmapatiënten, maar dat deze ook verschillen tussen patiënten met mild tot matig astma en die van ernstig astma. Daarbij bleek dat het gebruik van geïnhaleerde ontstekingsremmende corticosteroïden ook de reacties van eosinofielen beïnvloedt. Het bleek verder dat de eosinofiele granulocyten van patiënten die geen medicatie gebruiken slechter virus kunnen binden in vergelijking met die cellen van patiënten die wel medicijnen gebruiken. De medicatie bevorderde niet alleen virus binding maar zorgt ook voor minder actieve cellen. Blijkbaar moeten eosinofielen geactiveerd zijn om virus te kunnen binden, alhoewel te actief wel weer voor schade zorgt. PDE4 inhibitoren kunnen daarom een veelbelovend nieuw astmamedicatie zijn als onderhoudsmedicatie en ter preventie van exacerbaties.

In **hoofdstuk 6** wordt een patiënten studie beschreven van 83 patiënten met bronchiëctasieën. Bronchiëctasieën vertegenwoordigen een relatief zeldzame longziekte, die wel vaak samengaat met andere longziekten zoals astma, waarbij delen

van de lagere luchtwegen constant geïrriteerd en verwijd zijn. In deze studie wordt gekeken naar de ontstekingsprocessen en de rol van ontstekingscellen in relatie tot een antibioticum behandeling. In totaal kregen 43 patiënten behandeling met het antibioticum Azitromycine (AZM) en 40 patiënten een placebo behandeling. Twaalf maanden antibiotica behandeling resulteerde in een verminderd aantal exacerbaties in vergelijking met de controle behandeling. Elke 3 maanden werd sputum verzameld en hierin werd gekeken naar ontstekingsmediatoren en de activatie van eosinofielen. De klinische indicatoren en de ontstekingsprocessen in de patiënten voor de behandeling met antibioticum of placebo waren vergelijkbaar en werden niet beïnvloed door onderliggende ziekte zoals astma of chronisch obstructief longlijden (COPD). Ook het inflammatoire profiel is relatief niet echt anders in deze drie groepen. Wel konden wij in de ontstekingsprocessen goed onderscheiden wie vaker in de totale populatie en of in de groepen met astma of COPD een acute verslechtering (exacerbatie) hadden. In astma correleren exacerbaties met een ontstekingsbevorderend en neutrofiel profiel, terwijl wij dit bij COPD niet konden zeggen omdat het aantal patiënten met COPD te gering was. Helaas kunnen we geen duidelijk antwoord geven op het effect van AZM op de inflammatie, ook hiervoor hadden wij nog te geringe aantallen.

In **hoofdstuk 7**, laten we een nieuwe techniek zien om de relatieve eosinofiele en neutrofiele sputum tellingen te kunnen vervangen. Omdat de gouden standaardmethode arbeidsintensief en specialistisch is, hebben we een snelle eenvoudige methode ontwikkeld die minder ervaring vereist. Hierin hebben we “whole induced” sputum materiaal gebruikt, omdat deze meer plaveiselepitheel kunnen bevatten dan bijvoorbeeld geselecteerde pluggen. Boven alle verwachtingen, hadden zelfs sputa met meer dan 80% plaveiselepitheel nog steeds EPO/MPO waarden (specifieke kenmerken van eosinofiele en neutrofiele granulocyten). Deze EPO/MPO waarden correleerde goed met de relatieve aantallen eosinofiele en neutrofiel granulocyten. Dit in combinatie met het feit dat deze EPO/MPO ratio onaangedaan is door de aanwezigheid van andere cellen en oplosbare componenten zoals hemoglobine, zorgt ervoor dat deze nieuwe techniek meer accuraat de eosinofiele of neutrofiele inflammatie weergeeft. Wij vermoeden dat dit vooral een bruikbare techniek is voor het analyseren van patiënten die veel sputum opgeven, zoals bij COPD en bij bronchiëctasieën.

PHD PORTFOLIO

PhD Student

Yanaika Shari Sabogal Piñeros
Born 22th of March 1990, Purmerend, the Netherlands
PhD supervisors: Dr. R. Lutter and Prof. Dr. P.J. Sterk
PhD period: August 2013 – november 2019
Total ECTS obtained: 56

Training and courses

♥ Oral presentation	– 2014 – 0.8 ECTS
♥ Infectious Diseases	– 2013 – 1.3 ECTS
♥ Practical Biostatistics	– 2014 – 1.1 ECTS
♥ Veneuze bloedafname	– 2014 – 1.5 ECTS
♥ Basic laboratory safety	– 2013 – 0.4 ECTS
♥ Advanced Immunology	– 2015 – 2.9 ECTS
♥ Symposium Update GCP	– 2016 – 0.5 ECTS
♥ Scientific Writing in English	– 2013 – 0.4 ECTS
♥ Expert Management of Medical Literature: Endnote	– 2014 – 0.1 ECTS
♥ BROK: Basiscursus Regelgeving Klinisch Onderzoek	– 2013 – 0.9 ECTS
♥ CRA-Track: basis track voor Clinical Research Associate	– 2018 – 2.0 ECTS
♥ Merck/CNTO: Adverse experience reporting training; ICH-GCP e-Learning; Informed consent process e-Learning Course; IATA dangerous goods; BROK-update symposium 2016/2019; BIOKE workshops IF and Flow cytometry; open clinica training; EPIC	–13/18 – 1.1 ECTS

Seminars

♥ Yearly retreat: EXIM	– 2013/19 – 2 ECTS
♥ Journal Club: ALLERGY & LUNG	– 2013/17 – 3 ECTS
♥ Weekly department seminars: AIM	– 2013/16 – 3 ECTS
♥ Weekly department seminars: EXIM	– 2013/19 – 5 ECTS
♥ Weekly department seminars: LUNG	– 2013/19 – 5 ECTS

Conferences

♥ IES	– Oxford; UK	– 2013 – 0.75 ECTS
♥ NVVI	– Noordwijkerhout; NL	– 2013 – 0.50 ECTS
♥ ERS	– Estoril; Portugal	– 2014 – 0.75 ECTS

- ESCI – Utrecht; NL – 2014 – 0.75 ECTS
- NVVI – Kaatsheuvel; NL – 2014 – 0.75 ECTS
- Longdagen – Utrecht; NL – 2015 – 0.50 ECTS
- ESCI – Cluj Napoca; Romania – 2015 – 0.75 ECTS
- IES – Chicago; US – 2015 – 1.00 ECTS
- NVVI – Noordwijkerhout; NL – 2015 – 0.75 ECTS
- ERS – Estoril; Portugal – 2016 – 0.50 ECTS
- NVVI/BSI – Liverpool; UK – 2016 – 1.00 ECTS
- WIRM – Davos; Switzerland – 2017 – 0.75 ECTS
- ERS – Estoril; Portugal – 2019 – 0.50 ECTS

Presentations

- NVVI - Poster Presentation- Do human eosinophils phagocytose respiratory viruses? – 2013 – 0.5 ECTS
- ERS - Poster Presentation - An important role for eosinophils in the antiviral immune response. – 2014 – 0.5 ECTS
- ESCI - Poster Presentation - Can human eosinophils phagocytose and degrade respiratory viruses? – 2014 – 0.5 ECTS
- Triple I PhD retreat – Oral Presentation - An anti-viral role for eosinophils - Direct interaction between peripheral eosinophils and respiratory viruses. – 2014 – 1.0 ECTS
- NVVI - Poster Presentation - Eosinophils display direct antiviral activity to respiratory viruses. – 2014 – 0.5 ECTS
- Longdagen – Oral Presentation – The two faces of eosinophils in virus-induced asthma exacerbations. – 2015 – 1.0 ECTS
- ESCI – Oral Presentation – The two faces of eosinophils in virus-induced asthma exacerbations. – 2015 – 1.0 ECTS
- IES – Poster Presentation – Eosinophils display damaging and protective properties upon viral exposure; its relevance in virus-induced loss of asthma control. – 2015 – 0.5 ECTS
- NVVI – Poster Presentation – Eosinophils rapidly degrade respiratory viruses and are mildly activated; its relevance in virus-induced loss of asthma control. – 2015 – 0.5 ECTS
- ERS – Poster Presentation – A dual role for eosinophils upon viral exposure; its relevance in virus-induced loss of asthma control. – 2016 – 0.5 ECTS
- NVVI/BSI – Oral Presentation – Defective internalization and inactivation of virus by eosinophils in asthma patients. – 2016 – 1.0 ECTS

- WIRM – Poster Presentation – Defective internalization and inactivation of virus by eosinophils in asthma patients. – 2017 – 0.5 ECTS
- ATS – Poster Presentation – Anti-IL5 Treatment Alters Eosinophil Responses to a Rhinovirus-16 Challenge in Mild Asthma Patients and Also that of Neutrophils, Macrophages and B Cells. – 2018 – 0.5 ECTS
- ERS – Oral presentation Young Investigator Session - Anti-IL5 in mild asthma alters rhinovirus-induced macrophage, B cell and neutrophil responses – 2019 – 1.0 ECTS

Supervising

♥	Master Student:	Nigel Jansen – The effect of eosinophil priming on the uptake of respiratory viruses.	Jan – May 2014 – 1 ECTS
♥	Master Student; internship:	Olga Snip – Does blocking of phosphodiesterase 4 activity enhance anti-viral activity by eosinophils?	Feb – Aug 2014 – 2 ECTS
♥	Master Student; internship:	Esmée P. Hoefsmit – Mechanisms underlying the anti- viral activity of eosinophils.	Jan – Aug 2016 – 2 ECTS
♥	Master Student; internship:	Suzanne van Gelderen – Mechanisms underlying RSV binding by eosinophils.	Nov – Apr '16/17 – 2 ECTS

- Chairing session asthma and allergy at ERS 2016 – Estoril
- Chairing poster session at ERS 2019 – Estoril
- Organizing BROK/GCP symposium 2019 – Amsterdam

Awards and Prizes

- ESCI Poster Award 2014 – Utrecht
- ESCI Oral Communication Award 2015 – Cluj Napoca
- ERS travel grant 2016 – Estoril
- ERS travel grant 2019 – Estoril
- Nominated for Young Investigator Award 2019 – Estoril

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