Potent Anti-Inflammatory Effects of Tetracyclines on Human Eosinophils

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Eosinophils are potent pro-inflammatory cells. Not only in allergic diseases but also in other diseases there is a need for treatment strategies to induce resolution of eosinophil-mediated inflammation. During the last years beneficial non-antibiotic activities of tetracyclines (TCNs) have been shown in different diseases in which eosinophils play a role, for example, asthma and bullous pemphigoid. The working mechanism of these effects remains to be clarified. Aim of the present study was to investigate the effects of TCNs on eosinophils. Flow cytometry analysis of apoptosis, mitochondrial membrane potential, activation of caspases, intracellular H$_2$O$_2$ and calcium, surface expression of eosinophil activation markers was performed in highly purified peripheral blood eosinophils of non-atopic donors. Tetracycline hydrochloride, minocycline and doxycycline significantly induced eosinophil apoptosis. All TCNs were able to significantly overcome the strong survival enhancing effects of pro-eosinophilic cytokines and staphylococcus aureus enterotoxins. Tetracycline hydrochloride induced eosinophil apoptosis was accompanied by intracellular production of hydrogen peroxide, loss of mitochondrial membrane potential and activation of caspases. Moreover, tetracycline hydrochloride significantly down regulated eosinophil surface expression of CD9 and CD45, and of the activation markers CD11b and CD69, but not of CD54, CD63, or CD95. Our data, probably for the first time, point to a potent anti-inflammatory role of TCNs on eosinophils.

Keywords: eosinophils, apoptosis, Staphylococcus enterotoxin, allergic inflammation, tetracycline

INTRODUCTION

Eosinophils are multifunctional leukocytes and by modulating innate and adaptive immunity, they play a pro-inflammatory key role in several diseases (1) including parasitic infections (2) allergic diseases such as atopic dermatitis and inhalant allergy (3–7), but also autoimmune diseases such as bullous pemphigoid (8), esophageal (9), and gastrointestinal disorders (10), mental disorders (11) and cancer (12–14). The downregulation of pro-inflammatory activities and induction of apoptosis in eosinophils promotes the resolution of inflammation. Until now, only few promoters of eosinophil apoptosis aside from glucocorticosteroids (15, 16) have been described, for example, theophylline (17) transforming growth factor-β (18, 19), abrogation of Fas/CD95 by its ligand or a monoclonal antibody (mAb) (20, 21) or CD69 perturbation with mAb (22). Unfortunately, most of these are not available as prescription drugs or have potent adverse effects.
Tetracyclines (TCNs) were discovered in the 1940s and are broad-spectrum antibiotics that act as such at the ribosomal level where they interfere with protein synthesis (23). Dermatologists are using TCNs since the 1950s to treat disorders that do not necessarily have an infectious etiology (24). In recent years, several studies have conclusively reported non-antibiotic activities of TCNs including anti-inflammatory, immune-modulating and neuroprotective properties and many clinical trials are ongoing over a wide range of diseases including dermatological diseases, behavior and mental disorders, immune system disorders, cardiovascular diseases, and cancer (25). For example, the BLISTER study has shown that starting patients on doxycycline is non-inferior to standard treatment with oral prednisolone for short-term blister control in bullous pemphigoid and significantly safer in the long-term (26). A recent systematic review and network meta-analysis concluded that combined doxycycline and nicotinamides are a safer and more effective option for extensive bullous pemphigoid patients as compared to usual use of systemic steroids and that minocycline may be a safer promising option for renal impairment patients (27). The beneficial non-antibiotic properties of TCNs in bullous pemphigoid and other diseases are far from being clear. The prominent deleterious role of eosinophils in bullous pemphigoid is well-established (28–31).

Therefore, our aim was to investigate whether TCNs modulate eosinophil function and survival.

**MATERIALS AND METHODS**

**Reagents**

If not otherwise stated, reagents were obtained from Sigma-Aldrich Chemicals, Schnelldorf, Germany. Pro-eosinophilic cytokine mix consisted of Interleukin (IL)-3, IL-5, and GM-CSF, 10 ng/ml each (R&D Systems, Wiesbaden, Germany). *Staphylococcus aureus* enterotoxins (SE) of maximal purity (without LPS contamination), SEA, SEB, and SEC were obtained from Toxin Technology, Inc (Sarasota, Fla).

**Purification of Eosinophils**

Peripheral blood eosinophils from healthy non-atopic volunteers after informed consent (approved by the ethics committee of the Hannover Medical School) were either separated by Ficoll density gradient centrifugation and an improved immunomagnetic negative selection procedure using anti CD16 antibody-coated Dynabeads (Dynal A.S., Oslo, Norway) as described in detail (32–34) or were isolated using a magnetic cell separation system (MACS) according to the instructions of the manufacturer (Milteny Biotec, Bergisch Gladbach, Germany). Purity and viability were 96% or greater after isolation, as assessed by Kimura staining and trypan blue dye exclusion, respectively.

**Modified Nicoletti’s Protocol and Mitochondrial Membrane Potential**

The proportion of eosinophils displaying a hypodiploid DNA peak was determined using a modification of the protocol of Nicoletti as described (35–37). In brief, 1 × 10⁶ eosinophils were resuspended in 200 µl of hypotonic fluorochrome solution (propidium iodide, 50 µg/ml in 0.1% sodium citrate plus 0.1% Triton X-100) and incubated for 2 h at 4°C. Apoptotic eosinophil nuclei were distinguished by their hypodiploid DNA content from the diploid DNA content of normal eosinophil nuclei. For inhibition of apoptosis eosinophils were pre-incubated for 2 h at 37°C with 50 µM of pan-caspase inhibitor (zVAD-fmk; R&D Systems, MN, USA) and then washed and stimulated with tetracycline hydrochloride (5 × 10⁻⁴ M and 10⁻⁴ M), and dexamethasone (10⁻⁶ M) for 24 h. For mitochondrial membrane potential detection 1 × 10⁶ eosinophils were incubated with 10 mM JC-1 (Molecular Probes, Eugene, OR) at room temperature for 20 min, centrifuged, washed twice with 10 x assay buffer, and analyzed by flow cytometry. In healthy cells with high mitochondrial membrane potential (∆Ψm), JC-1 spontaneously forms complexes known as J-aggregates with intense red fluorescence 2 (∼590 nm). In apoptotic cells with low ∆Ψm, JC-1 remains in the monomeric form, which shows only green fluorescence 1 (∼525 nm). The ratio of green to red fluorescence is dependent only on the membrane potential.

**Flow Cytometric Analysis of Intracellular H₂O₂, Calcium Mobilization and Surface Antigens**

To measure changes in intracellular H₂O₂, we used the oxidation-sensitive fluorescent probe dihydrorhodamine (DHR; Molecular Probes, Eugene, Oregon, U.S.A.). Eosinophils (1 × 10⁶/ml) were incubated with control medium, dexamethasone (10⁻⁶ M) or tetracycline hydrochloride as indicated for 2 and 24 h at 37°C. Thereafter the cells were labeled with 1 µM DHR for another 10 min at 37°C. PMA (10⁻⁷ M) was used as positive control. Fluo-4 AM is essentially non-fluorescent in the absence of Ca²⁺ and exhibits an increase in fluorescence emission upon binding Ca²⁺. 1 × 10⁶ eosinophils/ml in calcium influx buffer (PBS supplemented with 1 mM calcium chloride, 1 mM magnesium chloride, and 1% bovine serum albumin) were loaded with 5 µM Fluo-4 AM (Molecular Probes, Karlsruhe, Germany) for 30 min at 37°C in the dark. After washing and centrifugation measurement was performed in the FLUOstar plate reader (BMG Lab Technologies, Offenburg, Germany). After 5 s stimulation with tetracycline hydrochloride (5 × 10⁻⁴ M), C5a (10⁻⁸ M, positive control), or medium were done and changes in cellular fluorescence (excitation, 485 nm; emission, 520 nm) was recorded per second for 50 s. To characterize the effect of tetracycline hydrochloride on surface antigen expression single color immunofluorescence technique was performed as described previously (33). Surface antigen staining was performed after stimulation of eosinophils with control medium, IL-3 (10 ng/ml), dexamethasone (10⁻⁹ M) or tetracycline hydrochloride (10⁻³ M, 5 × 10⁻⁴ M, 10⁻⁴ M) for 24 and 48 h.

**Statistics**

Unless otherwise stated all data are presented as mean ± SEM. To see if the effect of stimulation was significant, paired t-test or Wilcoxon signed Rank Test were used, depending on the distribution of the data, a statistical software package (SigmaStat).
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FIGURE 1 | Tetracycline hydrochloride, minocycline and doxycycline induced eosinophil apoptosis. Stimulation of eosinophils with indicated TCNs at $10^{-4}$ M up to $10^{-3}$ M for 24 h (■) and 48 h (▲) ($p < 0.05$ up to $p < 0.001$ vs. medium control = 0). Mean percentage of apoptotic nuclei ± SEM ($n = 9$). Representative histogram analysis.

**RESULTS**

**TCNs Induced Caspase-Dependent Eosinophil ApoptosisPreceded by Mitochondrial Damage**

Tetracycline hydrochloride, doxycycline, and minocycline significantly ($p < 0.05$ up to $p < 0.001$, $n = 9$) and dose-, and time-dependently induced eosinophil apoptosis after 24 and 48 h incubation (Figure 1). TCNs did not induce necrosis as assessed by trypan blue dye exclusion (tetracycline hydrochloride $10^{-3}$ M: 1% dead cells after 48 h; minocycline $10^{-3}$ M 14% dead cells after 48 h; doxycycline: 10% with $5 \times 10^{-4}$ M and 2% with $10^{-4}$ M) except for higher concentrations of doxycycline (39% dead cells with $10^{-3}$ M). As tetracycline hydrochloride induced fewest necrotic eosinophils and significantly and dose-dependently induced apoptosis, we chose this TCNs for further experiments. Forty eight hours co-stimulation with the pan-caspase inhibitor zVAD-fmk abrogated tetracycline hydrochloride ($5 \times 10^{-4}$ M) induced apoptosis (Figure 2) suggesting that tetracycline hydrochloride caused caspase activation downstream of mitochondrial injury. These results implicate stress-induced signaling pathways in tetracycline-mediated apoptosis. Stimulation with tetracycline hydrochloride at $5 \times 10^{-4}$ M resulted in a significant loss of mitochondrial membrane potential ($\Delta \Psi_m$; $p = 0.005$ compared to medium, $n = 5$, Figure 3A). The representative dot plot (Figure 3B) demonstrates the increase in cell numbers with decreased red fluorescence 2.

**TCNs Inhibited Prolongation of Eosinophil Survival Mediated by staphylococcus aureus Enterotoxins or IL-3, IL-5, and GM-CSF**

All TCNs, i.e., tetracycline hydrochloride, doxycycline, minocycline, at $5 \times 10^{-4}$ M and $10^{-3}$ M were able to...
Calcium Influx in Eosinophils

Tetracycline Hydrochloride Induced Calcium Influx in Eosinophils

Eosinophils were stimulated with tetracycline hydrochloride (5 × 10⁻⁴ M) and tetracycline hydrochloride for 24 and 48 h was able to significantly downregulate the expression of the eosinophil activation markers CD11b and CD69 (p < 0.05 up to p < 0.001; Figure 7).

DISCUSSION

Unspecified non-antibiotic activities have been attributed to the beneficial effects of TCNs in several diseases. Some of these diseases are characterized by eosinophil-induced inflammation, for example asthma and bullous pemphigoid. Aim of this study was to investigate whether TCNs modulate eosinophil function and survival. Our data for the first time clearly show that TCNs, i.e., tetracycline hydrochloride, minocycline and doxycycline, significantly and dose-dependently induce eosinophil apoptosis. In contrast to necrosis, apoptosis represents a major physiologic mechanism that enables clearance of cells by phagocytosis with no or minimal damage to surrounding tissues (38). We and others have shown that delayed eosinophil apoptosis is deleterious in various allergic diseases such as atopic dermatitis, allergic rhinitis, and asthma (4, 6, 39–41). The development of drugs targeting eosinophil apoptosis is one possible strategy for the therapy of eosinophil-driven diseases.

There's some debate on the role of antibiotic therapy in the treatment of allergic disorders with concomitant bacterial infections (42, 43). Although antibiotic treatment has different outcomes in asthma, chronic rhinitis, and nasal polyposis, the impact of S. aureus enterotoxins in allergic diseases is increasing (44–52). Staphylococcus aureus enterotoxins are thought to act as superantigens, inducing production of polyclonal IgE via B-cell and T-cell activation, and triggering release of inflammatory mediators (50). Previously, we demonstrated that S. aureus enterotoxins are potent inhibitors of eosinophil apoptosis in atopic dermatitis (33). Therefore, we performed a series of experiments that demonstrated, that all TCNs significantly suppressed the survival-prolonging effect of SEA, SEB, and SEC. In addition, the potency of TCNs was confirmed by demonstrating, that tetracycline hydrochloride abrogated the well-known survival-enhancing effect of a mixture of pro-eosinophilic cytokines (53), namely IL-3, IL-5, and GM-CSF. High concentrations of systemic and also inhaled glucocorticoids have been reported to partly reverse IL-5-afforded survival, but the effect of steroids fell off as the concentration of IL-5 increased (16, 54). It has been shown that 1 µM dexamethasone was reversed by low concentrations of GM-CSF (10 ng/ml) or IL-3 (3 ng/ml) (15). In contrast, in this study tetracycline hydrochloride induced eosinophil apoptosis even in the presence of high concentrations of a mixture of IL-3, IL-5, and GM-CSF (10 ng/ml each). Tetracycline hydrochloride was chosen for further experiments as its stimulation had demonstrated fewest necrotic eosinophils compared to minocycline and doxycycline.

Caspases are activated in the interior of cells during the process to apoptosis and cleave specific death substrates. At
FIGURE 4 | TCNs overcame the survival prolonging effects of SEA, SEB, SEC, and of key pro-survival eosinophil cytokines, i.e., a mixture of IL-3, IL-5, and GM-CSF (ProEo). (A) Co-incubation of $5 \times 10^{-4}$ M or $10^{-3}$ M tetracycline hydrochloride (Tetra), doxycycline (Doxi), or minocycline (Mino) with SEA, SEB, and SEC (5 μg/ml each; $p < 0.05$ up to $p < 0.001$ vs. medium control $= 0$). Data are presented as percentage of apoptotic nuclei ± SEM ($n = 6$). (B) Tetra partly abrogated the effect of ProEo. Mean percentage of apoptotic nuclei ± SEM ($n = 5$) after stimulation for 24 h (unfilled bars) and 48 h (striped bars). $P < 0.05$ up to $p < 0.001$.

least IL-5 is known to prolong eosinophil survival via inhibition of caspases (55). Using a pan-caspase inhibitor (zVAD-fmk) our experimental data showed that tetracycline hydrochloride-induced eosinophil apoptosis was caspase dependent.

FIGURE 5 | Tetracycline hydrochloride induced intracellular hydrogen peroxide production. In contrast to dexamethasone $10^{-6}$ M (Dex) tetracycline hydrochloride (Tetra) induced apoptosis was accompanied by a significant intracellular production of H$_2$O$_2$ after 2 h (unfilled bars) and 24 h (striped bars). Dihydrorhodamine assay, mean fluorescence intensity (MFI) ± SEM ($n = 7$). **$p < 0.01$, ***$p < 0.001$.

Aside from inhibition of matrix metalloproteinases (23) some tetracycline analogues have been reported to act as inducers of apoptosis in various cancer cells (56–60). However, the precise apoptotic mechanisms are far from being understood. In this study, we showed that tetracycline hydrochloride produced an early increase in loss of mitochondrial membrane potential.
similar changes of ΔΨm have been demonstrated in other cell types (61). Additionally, we found a significant upregulation of eosinophil intracellular hydrogen peroxide production 2 and 24 h after stimulation with tetracycline hydrochloride. These results clearly indicate a potent role of stress in the induction of TCNs induced eosinophil apoptosis. This is in contrast to dexamethasone induced apoptosis that is not associated with an increase of intracellular hydrogen peroxide [our data and (62)]. We and others have shown an important role of oxygen-dependent mechanisms in the regulation of eosinophil survival and apoptosis (32). Whilst tetracycline hydrochloride induced intracellular hydrogen peroxide it is tempting to speculate that TCNs may induce apoptosis by disturbing the eosinophil oxidant-antioxidant balance (32). Moreover, we demonstrated that tetracycline hydrochloride immediately increased intracellular calcium and eosinophil apoptosis has been shown to be mediated by calpains that are activated by increased intracellular calcium (63).

Addressing modulation of eosinophil surface antigen expression we found that tetracycline hydrochloride significantly downregulated activation markers such as CD11b and CD69, in addition to CD9 and CD45. We previously demonstrated that these markers are up-regulated on eosinophils by S. aureus enterotoxins (33). Interestingly, in that previous study the apoptosis-inducing corticosteroid dexamethasone downregulated CD11b and CD45, but resulted in a significant increase of CD54 and CD69 (33). All surface markers that were down-regulated by tetracycline hydrochloride in this study have been implicated in the regulation of eosinophil apoptosis (64–66). These down-regulating properties of important apoptosis-regulating and activation markers on eosinophils underline the anti-inflammatory potential of TCNs.

Our data demonstrating potent anti-inflammatory effects of TCNs on eosinophils may provide a new paradigm for the choice of antibiotic treatment—if needed—in diseases associated with eosinophil inflammation. Moreover, in the future, the non-antibiotic activities of TCNs might be specifically utilized. Indeed, recent findings demonstrated that TCNs reduced airway inflammation and hyperresponsiveness in asthma animal models and that in humans, they have steroid-sparing properties in moderate persistent and “difficult to treat” asthma (51, 67–71). For the first time our results may provide an explanation for the reported non-antibiotic therapeutic effect of TCNs in bullous pemphigoid, a blistering disease with a prominent role of eosinophils that are increased in peripheral blood and in blister fluids (72, 73).

In conclusion, key findings from this study demonstrate that TCNs are very potent inducers of mitochondria-mediated and caspase-dependent apoptosis in eosinophils, down-regulate eosinophil activation markers and other surface molecules, and are even capable to overcome the effect of potent survival-enhancing cytokines such as IL-3, IL-5, and GM-CSF and S. aureus exotoxins. Clinically, TCNs might have less severe side effects compared to corticosteroids, which actually, are used as therapeutic option in some diseases associated with eosinophilia. Our results indicate new non-antibiotic anti-inflammatory effects of TCNs, which should be addressed in more detail in future studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Hannover Medical School. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MG and BW conceived and planned the experiments, processed the experimental data, and performed the analysis. MG carried out the laboratory works. DW contributed to sample preparation. BW designed the figures and took the lead in writing the manuscript with support from DW and AK. All authors provided critical feedback and helped shape the research, analysis, and manuscript.
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REFERENCES

1. Hogan SP, Rosenberg HF, Moqbel R, Phipps P, Foster PS, Lacy P, et al. Eosinophils: biological properties and role in health and disease. Clin Exp Allergy. (2008) 38:799–50. doi: 10.1111/j.1365-2222.2008.02958.x
2. Huang L, Appleton JA. Eosinophils in helminth infection: defenders and dupes. Trends Parasitol. (2016) 32:798–807. doi: 10.1016/j.pt.2016.05.004
3. Kapp A, Czech W, Krutmann J, Sch pf F. Eosinophil cationic protein in sera of patients with atopic dermatitis. J Am Acad Dermatol. (1991) 24:555–8. doi: 10.1016/0190-9622(91)70081-C
4. Wedi B, Raap U, Lewrick H, Kapp A. Delayed eosinophil programmed cell death in vitro: a common feature of inflammatory and extrinsic and intrinsic atopic dermatitis. J Allergy Clin Immunol. (1997) 100:536–43. doi: 10.1016/S0091-6749(97)70147-7
5. Vignola AM, Chanez P, Chiappara G, Siena L, Merendino A, Reina C, et al. Evaluation of apoptosis of eosinophils, macrophages, and T lymphocytes in mucosal biopsy specimens of patients with asthma and chronic bronchitis. J Allergy Clin Immunol. (1999) 103:563–73. doi: 10.1016/S0091-6749(99)70225-3
6. Woolley KL, Gibson PG, Carty K, Wilson AJ, Twaddell SH, Woolley MJ. Eosinophil apoptosis and the resolution of airway inflammation in asthma. Am J Respir Crit Care Med. (1996) 154:237–43. doi: 10.1164/ajrccm.154.3.8670086
7. Kankaanranta H, Lindsay MA, Giembycz MA, Zhang X, Moilanen E, Barnes PJ. Delayed eosinophil apoptosis in asthma. J Allergy Clin Immunol. (2000) 106:787–83. doi: 10.1067/mca.2000.107038
8. Long H, Zhang G, Wang L, Lu Q. Eosinophilic skin diseases: a comprehensive review. Clin Rev Allergy Immunol. (2016) 50:189–213. doi: 10.1007/s12016-015-0458-8
9. Biedermann L, Straumann A, Greuter T, Schreiner P. Eosinophilic esophagitis-established facts and new horizons. Semin Immunopathol. (2021) 43:319–35. doi: 10.1007/s00281-021-00855-y
10. Mehta P, Furuta GT. Eosinophils in gastrointestinal disorders: eosinophil gastrointestinal diseases, celiac disease, inflammatory bowel diseases, and parasitic infections. Immunol Allergy Clin North Am. (2015) 35:413–37. doi: 10.1016/j.iac.2015.04.003
11. Dean OM, Data-Franco J, Giorlando F, Berk M. Minocycline: a common feature of inhalant allergy and extrinsic allergic inflammatory reactions. J Allergy Clin Immunol. (2018) 109:477–8. doi: 10.1016/j.jaci.2017.07.022
12. Lee D, Ahn TB. Central nervous system involvement of hypereosinophilic syndrome: a report of 10 cases and a literature review. J Neurol Sci. (2017) 37:446–71. doi: 10.1016/j.jns.2017.01.026
13. Simon D, Borradori L, Simon HU. Eosinophils as putative therapeutic targets in bullous pemphigoid. Exp Dermatol. (2017) 26:1187–92. doi: 10.1111/exd.13416
14. Rüdrich U, Gehring M, Papakonstantinou E, Kapp A, et al. Eosinophils are a major source of interleukin-31 in bullous pemphigoid. J Allergy Clin Immunol. (2018) 141:1877–86. doi: 10.1016/j.jaci.2018.01.070
15. Roos CAT, Brandt IM, Houbiers JK, Keirse MJ, et al. Staphylococcal toxins exert proinflammatory effects through inhibition of eosinophil apoptosis, increased surface antigen expression (CD11b, CD45, CD54, and CD69), and enhanced cytokine-activated oxidative burst, thereby triggering allergic inflammatory reactions. J Allergy Clin Immunol. (2002) 109:77–84. doi: 10.1067/mcl.2002.121702
16. Schefzyk M, Bruder M, Schmiedl A, Stephan M, Kapp A, Wedi B, et al. Eosinophil granulocytes: functional differences of a new isolation kit compared to the isolation with anti-CD16-conjugated Microbeads. Exp Dermatol. (2009) 18:653–5. doi: 10.1111/j.1600-0625.2008.00824.x

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35. Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C. A rapid and non-invasive method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Methods. (1991) 139:271–9. doi: 10.1016/0022-1759(91)90198-O

36. Hebestreit H, Dibbert B, Balatti I, Braun D, Schapowal A, Blaser K, et al. Disruption of FAS receptor signaling by nitric oxide in eosinophils. J Exp Med. (1998) 187:415–25. doi: 10.1084/jem.187.3.415

37. Hebestreit H, Youssef S, Balatti I, Weber M, Crameri R, Simon D, et al. Expression and function of the FAS receptor on human blood and tissue eosinophils. Eur J Immunol. (1996) 26:1775–80. doi: 10.1002/eji.1830260817

38. D’ArCY MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int. (2019) 43:582–92. doi: 10.1002/cbin.11137

39. Gleich GJ. Mechanisms of eosinophil-associated inflammation. J Allergy Clin Immunol. (2000) 105:651–65. doi: 10.1016/mia.2000.105712

40. Raap U, Golz C, Deneka N, Bruder M, Renz H, Kapp A, et al. Brain-derived neurotrophic factor is increased in atopic dermatitis and modulates eosinophil functions compared with that seen in nonatopic subjects. J Allergy Clin Immunol. (2005) 115:1268–75. doi: 10.1016/j.jaci.2005.02.007

41. Wardlaw AJ, Moqbel R, Kay AB. Eosinophils: biology and role in disease. Adv Immunol. (1995) 60:151–266. doi: 10.1016/S0065-2776(08)60586-8

42. Boguniewicz M, Sampson H, Leung SB, Harbeck R, Leung DY. Effects of Staphylococcus aureus enterotoxins in asthma: current knowledge. J Allergy Clin Immunol. (2018) 143:207–16. doi: 10.1016/j.jaci.2003.12.012

43. Onoda T, Ono T, Dhar DK, Yamanoi A, Fujii T, Nagase N. Doxycycline inhibits cell proliferation and invasive potential: combination therapy with cyclo-oxygenase-2 inhibitor in human colorectal cancer cells. J Lab Clin Med. (2004) 143:207–16. doi: 10.1016/j.lab.2003.12.012

44. Blaylock MG, Sexton DW, Walsh GM. Gutation of CD45 and the isoforms CD45RA and CD45RB accelerates the rate of constitutive apoptosis in human eosinophils. J Allergy Clin Immunol. (2004) 106:S99–103. doi: 10.1067/mia.2000.10

45. J Allergy Clin Immunol. (2008) 7:237–9. doi: 10.2165/00128807-200607050-00001

46. Hellings PW, Hens JG, Meyts I, Van Zele T, Gevaert P. The role of other factors in atopic dermatitis. J Allergy Clin Immunol. (2001) 108:651–2. doi: 10.1067/mia.2001.118598

47. Breuer K, Häuser S, Kapp A, Werfel T. Staphylococcus aureus colonizing features and influence of an antibacterial treatment in adults with atopic dermatitis. Br J Dermatol. (2002) 147:55–61. doi: 10.1046/j.1365-2133.2002.04872.x

48. Cardona ID, Cho SH, Leung DY. Role of bacterial superantigens in atopic dermatitis: implications for future therapeutic strategies. Am J Clin Dermatol. (2006) 7:273–9. doi: 10.2165/00128807-200607050-00001

49. Boguniewicz M, Sampson H, Leung SB, Harbeck R, Leung DY. Effects of cefuroxime axetil on Staphylococcus aureus colonization and superantigen production in atopic dermatitis. J Allergy Clin Immunol. (2001) 108:651–2. doi: 10.1067/mia.2001.118598

50. Raap U, Golz C, Deneka N, Bruder M, Renz H, Kapp A, et al. Brain-derived neurotrophic factor is increased in atopic dermatitis and modulates eosinophil functions compared with that seen in nonatopic subjects. J Allergy Clin Immunol. (2005) 115:1268–75. doi: 10.1016/j.jaci.2005.02.007

51. Wardlaw AJ, Moqbel R, Kay AB. Eosinophils: biology and role in disease. Adv Immunol. (1995) 60:151–266. doi: 10.1016/S0065-2776(08)60586-8

52. Jorde I, Hildebrand CB, Kershaw O, Lücke E, Stegemann-Koniszewski S, et al. Tetracycline analogs modulate expression of IgE to nasal polyposis? J Allergy Clin Immunol. (2000) 105:651–63. doi: 10.1067/mai.2000.105712

53. Shen ZJ, Malter JS. Determinants of eosinophil survival and apoptotic cell death. Ann N Y Acad Sci. (2005) 104:1244–50. doi: 10.1016/S0091-6749(05)75794X

54. J Allergy Clin Immunol. (2008) 5:433–45. doi: 10.1111/j.1525-1594.2007.00472.x

55. Dewson G, Cohen GM, Wardlaw AJ. Interleukin-5 inhibits translocation of Bax to the mitochondria, cytochrome c release, and activation of caspases in human eosinophils. Blood. (2001) 98:2239–47. doi: 10.1182/blood.V98.7.2239

56. D’Agostino P, Ferla P, violin S, LoRusso M, Di Bella G, Caruso R, et al. Chemically modified tetracyclines induce cytotoxic effects against J774 tumour cell line by activating the apoptotic pathway. Int Immunopharmacol. (2003) 3:63–73. doi: 10.1016/S1576-6510(02)0013-8

57. Fife RS, Rougraff BT, Proctor C, Sledge GW, Jr. Inhibition of proliferation and induction of apoptosis by doxycycline in cultured human osteosarcoma cells. J Lab Clin Med. (1997) 130:530–4. doi: 10.1016/S0022-1737(97)90130-X

58. Iwasaki H, Inoue H, Mitsuke Y, Badran A, Ikegaya S, Ueda T. Doxycycline induces apoptosis by way of caspase-3 activation with inhibition of matrix metalloproteinase in human T lymphoblastic leukemia CCRF-CEM cells. J Lab Clin Med. (2002) 140:382–6. doi: 10.1067/mlc.2002.129308

59. Lokeshwar BL, Selzer MG, Zhu BQ, Block NL, Golub LM. Inhibition of cell proliferation, invasion, tumor growth and metastasis by an oral non-antimicrobial tetracycline analog (COL-3) in a metastatic prostate cancer model. Int J Cancer. (2002) 99:297–309. doi: 10.1002/jic.10168

60. Ilmarinen P, Moilanen E, Kankaanranta H. Regulation of spontaneous eosinophil apoptosis—a neglected area of importance. J Cell Death. (2014) 7:1–9. doi: 10.4137/JCD.S13588

61. Blaylock MG, Sexton DW, Walsh GM. Gutation of CD45 and the isoforms CD45RA and CD45RB accelerates the rate of constitutive apoptosis in human eosinophils. J Allergy Clin Immunol. (2004) 106:S99–103. doi: 10.1067/mia.2000.10

62. Daoud A, Gloria CJ, Tanningo G, Hammerschlag MR, Weiss S, Gelling M, et al. Minocycline treatment results in reduced oral steroid requirements in adult asthma. Allergy Asthma Proc. (2008) 29:286–94. doi: 10.2500/aap.2008.29.3121

63. Guexders MM, Bertholet P, Perin F, Rocks N, Maree R, Botta V, et al. A novel formulation of inhaled doxycycline reduces allergen-induced inflammation, hyperresponsiveness and remodeling by matrix metalloproteinases and cytokines modulation in a mouse model of asthma. Biochem Pharmacol. (2008) 75:514–26. doi: 10.1016/j.bcp.2007.09.012

64. Rempe S, Hayden YM, Robbins RA, Hoyt JC. Tetracyclines and pulmonary inflammation. Endocr Metab Immune Disord Drug Targets. (2007) 7:232–6. doi: 10.2174/187135007782794344

65. Waghshul FA, Brown DT, Schulke NM, Hahn DL. Outcomes of antibiotics in adults with “difficult to treat” asthma or the overlap syndrome. J Asthma Allergy. (2021) 14:703–12. doi: 10.2147/IAA.S31408

66. Chalmers JR, Wojnarowska F, Kirtschig G, Nunn AJ, Bratton DJ, Mason J, et al. A randomized controlled trial to compare the safety and effectiveness of doxycycline (200 mg daily) with oral prednisolone...
(0.5 mg kg(-1) daily) for initial treatment of bullous pemphigoid: a protocol for the Bullous Pemphigoid Steroids and Tetracyclines (BLISTER) Trial. Br J Dermatol. (2015) 173:227–34. doi: 10.1111/bjd.13729

73. Gounni Abdelilah S, Wellemans V, Agouli M, Guenounou M, Hamid Q, Beck LA, et al. Increased expression of Th2-associated chemokines in bullous pemphigoid disease. Role of eosinophils in the production and release of these chemokines. Clin Immunol. (2006) 120:220–31. doi: 10.1016/j.clim.2006.03.014

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