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What about the neutrophils phenotypes?

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Abstract

Human neutrophils are in the sights of immunology, since recent research has positioned them as new players in adaptive immunity besides innate immunity. These leukocytes not only limit their function to phagocytosis, production of reactive oxygen species and generation of neutrophil extracellular traps, but also modulate the immune response according to the microenvironment and the cell-cell interactions, such as interactions between neutrophils, T cells and macrophages. This review summarizes current polymorphonuclear neutrophils research on some phenotypes in scientific literature in murine and human models.

Introduction

Polymorphonuclear (PMN) neutrophils are leukocytes that not only limit their function to phagocytosis, production of reactive oxygen species (ROS) and generation of neutrophil extracellular traps (NETs), but also modulate the immune response according to the microenvironment where they are and according to the interaction they establish with others cell types. Neutrophils have a role in the activation and regulation of innate and adaptive immunity [1]. The variability of its functions according to its environment, physiological or pathological condition, and the expression of different surface markers have allowed to characterize different profiles of neutrophils in murine and human models.

Interaction between neutrophils, macrophages and T cells

In relation to the interaction between neutrophils and macrophages, PMNs contribute to the activation and recruitment of macrophages at the site of infection or in acute inflammation [2]. There is evidence of the existence of "proinflammatory" and "anti-inflammatory" neutrophil subsets that show a unique pattern of cytokine and chemokine production and they differ in receptor expression. It is possible that these "proinflammatory" and "anti-inflammatory" forms of neutrophils change the course of the immune response by inducing M1 or classically activated macrophages and M2 alternatively activated, respectively [3].

About possible interactions between T cells and neutrophils, the cytokines IL17A, IL17F secreted by Th17 cells (which play a prominent role in the immunity against extracellular bacteria and mycotic infections and have also been related to autoimmune phenomena) induce a rapid and massive infiltration of the affected tissue by the neutrophils. Reciprocally PMNs release of chemokines that attract T lymphocytes to the inflammation sites [4-6] and also cytokines that influence differentiation and proliferation of T cells [7-9]. Interestingly, a subset of neutrophils in human systemic inflammation inhibits T cell functions through Mac-1 [10]. PMN neutrophils can release extracellular traps (NETs) which trap and kill a variety of microbes, and they have been proposed with role in T cell sensitization (9). NETs have been implicated in cancer immunoediting [11]. This NETs are composed of chromatin, histone and granule proteins [12,13]. Recently has been reported costimulatory molecules CD80 and CD86 colocalized in NETs [14,15]. This finding would allow PMNs to exert function as APCs and modulatory functions of various subpopulations of T cells. Neutrophils and Th17 cells have been colocalized in tissues of the gut of patients with Crohn's disease and in synovial fluid of patients with rheumatoid arthritis, in tissues isolated from patients with asthma and in tissues infected with *Helicobacter pylori* [2]. Neutrophils isolated from synovial fluid of patients with rheumatoid arthritis express Class II Major Histocompatibility Complex (MHC) molecules [16].

Major histocompatibility complex class II (DR) antigen and costimulatory molecules are observed on in vitro and in vivo activated human PMN neutrophils [17]. Cytoplasmic reservoirs of costimulatory molecules B7: CD80 mainly within secretory vesicles and CD86 within secondary, azurophilic granules and secretory vesicles were described [17].

The initiation of adaptive immune responses requires the fundamental stage of antigen presentation to T lymphocytes and currently the area of contact between T cell and an antigen presenting cell is known as "immune synapse" (SI) [18-20]. In murine models, neutrophil differentiation to a hybrid cell population exhibiting a dual phenotype with neutrophil and dendritic cell function [21] was experimentally described. Other authors demonstrated Class II restricted antigens presentation [22,23]. Although some neutrophils have been described at sites of inflammation expressing MHC Class II molecules and T-cell costimulatory molecules in mice and humans, their origin, phenotype and function remain unclear [24].

It is known that PMN neutrophils are considered short-lived cells in humans, but in inflammation, they increase its longevity. In vitro studies on leukocyte interactions in samples from patients with serology positive for Chagas' disease, the extension of its longevity in cell culture was observed [25]. Recently has been reported costimulatory

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Table 1. Characteristics of phenotypes of PMN neutrophils.

Reference	Phenotypes	Specie	Characteristics
Iking-Konert, *et al.* [29]	PMN MHC II (+) CD80+, CD86+	Human	• Patients with Wegener's Granulomatosis • 5 to 20% of neutrophils are larger and granular, expressing CD80, CD86 and MHC II • Acquire characteristics of antigen presenting cells • Patients with clinically inactive disease, with low levels of MHC II and CD80 expression • High expression of CD86 regardless of disease activity • CD64 Expression • PMN MHC II (+) and autologous T cells co-cultured with antigen showed significant T cell proliferation
Cross, *et al.* [16]	SF neutrophils	Human	• Patients with rheumatoid arthritis (RA) • synovial fluid (SF) neutrophils express class II MHC. MHC II expression in synovial fluid (SF) neutrophils in cultures of 20 h, Blood neutrophil cultures from RA patients also expressed MHC II • 8-fold more MHC II expression in synovial fluid neutrophils of patients with RA, in the presence or absence of GM-CSF or IFN-gamma • CD80 or CD86 expression does not increase significantly
Tsuda, *et al.*[30]		Mice	• PMN subsets distinguished according to their (A) cytokine and chemokine production, (B) activation of macrophages, (C) TLRs expression, (D) surface antigen expression • PMN-N can be converted to PMN-I or PMN-II depending on host circumstances
	PMN-N	Mice	• Obtained from naive mice • No effect on macrophage activation • TLRs expression: TLR-2, TLR-4, TLR-9 • surface antigen expression CD49d (-), CD11b (-)
	PMN-I	Mice	• Obtained from methicillin-resistant *Staphylococcus aureus* resistant hosts • IL-12, CCL3 production • macrophage activation: Classically activated macrophages • TLRs expression: TLR-2, TLR-4, TLR-5, TLR-8 • surface antigen expression CD49d (+), CD11b (-)
	PMN-II	Mice	• Obtained from methicillin-resistant *Staphylococcus aureus* sensitive hosts • IL-10, CCL2 production • macrophage activation: Alternatively activated macrophages • TLRs expression: TLR-4, TLR-7, TLR-9 • surface antigen expression: CD49d (-), CD11b (+)
Sandilands, *et al*. [31]	Neutrophils P1, after CD11b-cross linking	Human	• MHC II increases its expression at 2-5 min • CD80 and CD86 do not significantly increase their expression
	Neutrophils P2, after CD11b-cross linking	Human	• Increase in cell size • High level of expression of neutrophil activation marker CD64 • CD80, CD86 and HLA-DR significantly increase their expression. CD86 does it at 120 minutes • Express significantly elevated levels of MHC II, its expression is comparable to the levels expressed in monocytes • CD80, its expression is significantly high compared to that of monocytes
Sandilands, *et al.* [17]	Neutrophils P1,	Human	• After stimulation with phorbol myristate acetate (PMA), CD11b-cross linking, lipopolysaccharide (LPS), formylated peptides (fMLP) • Smaller PMNs • CD80 significantly increases expression after cross linking • CD86 significantly increases expression • Without stimulation in synovial fluid of rheumatoid arthritis (RA) PMNs P1 were observed, but not in paired blood samples. Increase in cell-surface CD66 expression
	Neutrophils P2,	Human	• After stimulation with PMA, CD11b-cross linking, LPS, fMLP • Larger PMNs • The phenotype is observed in different percentage according to the activator: - PMA 50% - Cross linking 20% - LPS 24% - fMLP 16% - Not activated 1% • MHC II and CD80 increase significantly • CD86 increases significantly • Without stimulation in synovial fluid of rheumatoid arthritis (RA) PMNs P2 were observed, but not in paired blood samples. Increase in cell-surface CD66 expression
Culshaw, *et al.* [22]		Mice	• Murine neutrophils expressed MHC II at low concentrations compared to macrophages • Neutrophils pulsed with OVA323-339 for 2 hrs, then co-cultured with transgenic T lymphocytes for 72 h, induced T-cell proliferation and IL-2 and IFN-gamma production • Murine neutrophils present Class II restricted antigen

Hacbarth and Kajdacsy-Balla, [32] Denny, *et al.* [33] Carmona-Rivera and Kaplan, [34]	Low density granulocytes (LDGs) Low density neutrophils	Human	• in PBMCs • isolated from the peripheral blood mononuclear cell fractions, in SLE • High expression of CD15 (to differentiate from monocytes) • Low expression of CD14 (to differentiate from monocytes) • Express CD10 and CD16 • Do not express MHC II and CD86 • Express surface markers similar to mature PMN • They differ in nuclear morphology that is consistent with immature phenotype • Proinflammatory profile: - High secretion of TNF, IFN I and II - High ability to generate NETs
Abi Abdallah, *et al.* [35]		Mice	• Neutrophils co-cultured with purified CD4 T cells, expressed MHC II, CD80, CD86 • PMN preincubated with OVA, triggered T-cell proliferation • PMN-pulsed with OVA, increase IFN-gamma and IL-17 production by cocultured T cells
Clemmensen, *et al.* [36]	PMN OLFM4(+)	Human	• First identification of subset of human neutrophils based on the presence or absence of granule matrix protein. • OLFM4 suggested in interacting with Catepsin C • OLFM4 is expressed heterogeneously in circulating neutrophils 20-25% • OLFM4 colocalizes with - NGAL (azurophilic granules) • Do not colocalize with MPO • OLFM4 appears in myelocytes
Ostanin, *et al.* [23]	Neutrophils Gr-1+	Mice	• In mice with chronic colitis • Presence of mature and immature neutrophils Mac-1^{+}, Ly6C^{int}, Gr-1^{+} • Expression of CD86, MHC II in neutrophils of colon • Neutrophils induce T cell activation in a dependent MHC II manner
Cloke, *et al.* [37]	Low density granulocytes (LDGs)	Human	• Study in HIV patients (+) • They are colocalized with peripheral blood mononuclear cells (PMBCs) and no in fraction of red blood cells • They express different levels of phenotypic markers of neutrophils: increased levels of CD11b, CD15, CD33, CD66b, CD63, and decreased levels of CD16 and arginase 1. • They increase their frequency in patients with low number of CD4 T cells- • Express Arginasa, CD15 • No express CD14
	Normal density granulocytes (NDGs)	Human	• Sediment with red blood cells • Phenotype varies according to the severity of the disease.
Welin, *et al.* [38]	Neutrophil OLFM4(+)	Human	• OLFM4 up regulation in tumorigenesis • PMN OLFM4 (+) represent 10-30% of the circulating PMNs • There is no functional difference between PMN OLFM4 (+) and (-) • The subset OLFM4 (+) is also found in tissues, generally more activated than in blood • They also generate NETs and OLFM4 is detected in them
Matsushima, *et al.* [21]	Neutrophil-dendritic cell	Mice	• Neutrophils cultured with GM-CSF • Hybrid cells expressing PMN surface markers - Ly6G - L-selectin - CXCR2 • And express surface markers of dendritic cells - CD11c - MHC II - CD80 - CD86 • Exhibit functionality as APC and release IL-12 • Preserve phagocytic function and release of NETs
Geng, *et al.* [24]	Neutrophil-dendritic cell	Mice	• Neutrophils cultured with GM-CSF • Neutrophils detected in inflammatory conditions in the peritoneal cavity, skin, lung and lymph nodes • Hybrid cells expressing PMN surface markers - Ly6G - L-selectin - CXCR2 • And express surface markers of dendritic cells - CD11c - MHC II - CD80 - CD86 • Exhibit functionality as APC and release IL-12 • Preserve phagocytic function and release of NETs

Hao, *et al.* [39]	Immune suppressive neutrophils	Human	• Suppressor myeloid-derived suppressor cells (MDSCs) have been identified as tumor suppressor • They represent heterogeneous family of myeloid cells that suppress T cell activation • Immunophenotype - CD11c bright - CD62L dim - CD11b bright - CD16 bright • These PMNs could be essential for limiting T cell activation and maintaining tolerance in inflammatory conditions
Davey, *et al.* [40]	PMN antigen cross presenting cell	Human	• Cross talk between PMN and gamma delta T cells and invariant mucosal associated T cells (MAIT) were studied in vitro • PMN primed with unconventional T cells expressed - CD40 - CD64 - CD83 - HLA-DR - CD54 - HLA ABC • As well as indicators of inflammation - CXCR3 - CCR4 • Unconventional T cell primed PMN cross-present antigens to CD8+ T cells.
Amsalem, *et al.* [41]	Decidual neutrophils	Human	• Proangiogenic granulocytes • They express high levels of activation markers and proteins related to angiogenesis - VEGF A - ARG 1 - CCL2 • Similar to PMNs associated with tumors • A significantly increased proportion of CD45+ CD15+ neutrophils was present neutrophils in second as compared with first trimester decidua.
Ssemaganda, *et al.* [42]		Human	• Study in pregnant women
	NDGs	Human	• They are found in maternal blood and umbilical cord at the time of parturition • They are not in term placenta • NDGs with high expression of Arginasa in maternal blood and umbilical cord compared to LDGs • Low expression of CD63 (azurophile granule release marker) compared to LDGs • NDGs of maternal blood and umbilical cord with similar phenotype
	LDGs	Human	• They are found in maternal blood, placenta and umbilical cord • Low expression of Arginasa in LDGs of umbilical cord blood, maternal blood and placenta • LDGs with high expression of CD33 (marker of immature neutrophils) in placenta, maternal blood and umbilical cord • High expression of CD63, CD15 (expressed by mature neutrophils) and CD66b • No phenotypic differences in the LDGs of the 3 compartments, except CD66b, with less expression in placental LDGs compared to those of maternal blood and umbilical cord • Higher frequency of LDGs in umbilical cord blood compared to placenta and maternal blood
Fine, *et al.* [43]	Parainflammatory neutrophils “Para1”	Human	• Neutrophils of the oral cavity in state of health • Size and granularity profile similar to circulating neutrophils • Low ROS and NETs production compared to Para2
	Parainflammatory neutrophils “Para2”	Human	• Increased degranulation • High expression of activation markers - CD55 - CD63 • Low expression of inhibitory markers - CD170 - CD16
	Proinflammatory neutrophils	Human	• Increased degranulation • They produce more ROS than parainflammatory • Size and granularity profile similar to Para2 • Produce more NETs • High expression of activated CD
Rodriguez and Novak, 2016 [14]		Human	• PMN stimulated with LPS and OVA • Costimulatory molecules CD80 and CD86 colocalized in NETs
Wang *et al.,* 2017 [44]	Neutrophils PD- L1+	Human	• Tumor associated neutrophils • Human gastric cancer • CD54+ • high level immunosuppressive molecule programmed death-ligand 1 (PD-L1). • Tumour-infiltrating PD-L1+ neutrophils exhibit immunosuppressive function and are correlated with disease stage and poor patient survival

Zou, *et al.* 2017 [45]	Neutrophils N2	Mice	• IL-35 induces N2 PMNs • Protumor phenotype • Proangiogenic and immunosuppressive functions • Increased expression of MMP-9 and Bv-8 • Decreased expression of TRAIL

molecules and extracellular traps in patients with serology positive for Chagas' disease and that was related with autoimmunity phenomena [26]. Suppressive IL-10-producing neutrophils were found in this parasitosis in a murine model, reducing inflammation and mortality during T. cruzi infection through IL17RA-signaling [27].

About of the role of PMNs in tumor development, polarization of Tumor-Associated Neutrophil (TAN) was reported. "N1" phenotype (anti-tumorigenic)) express higher levels of proinflammatory cytokines, they are hypersegmented, and cytotoxic to tumor cells. TGF-β presence defines differentiation toward "N2" phenotype (pro-tumorigenic) [28].

This review attempts to synthesize the characteristics of PMN neutrophils, from some phenotypes observed in scientific works (Table 1).

Conclusion

Neutrophils are very interesting cells implicated in innate and adaptive immunity. There are many described phenotypes of PMN neutrophils, but there are heterogenea descriptions in literature, because parameters, methods, specie, tissue, biomarkers, are different. Anyway, these studies are very useful in the context of the one developed avoiding the extrapolation.

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To all authors of reviewed literature

Disclosure statement

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References

1. Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol*11: 519-531.[Crossref]
2. Kumar V, Sharma A (2010) Neutrophils: cinderella of innate immune system. *International Immunopharmacology*10: 1325-1334.
3. Kolaczkowska E, Kubes P (2013) Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol*13: 159-175.[Crossref]
4. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Bazzoni F, et al. (2000) The neutrophil as a cellular source of chemokines. *Immunol Rev* 177: 195-203.[Crossref]
5. Müller I, Munder M, Kropf P, Hänsch G M (2009) Polymorphonuclear neutrophils and T lymphocytes: strange bedfellows or brothers in arms.*Trends Immunol*30:522-530.
6. Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, et al. (2010) Evidence for cross-talk between human neutrophils and Th17 cell. *Blood* 115: 335-343.
7. Denkers EY, Del Rio L, Bennouna S (2003) Neutrophil production of IL-12 and other cytokines during microbial infection. *ChemImmunol Allergy*83: 95-114.
8. Thewissen M, Damoiseaux J, van de Gaar J, Tervaert JW (2011) Neutrophils and T cells: bidirectional effects and functional interferences. *MolImmunol*48: 2094-2101.[Crossref]
9. Tillack K, Breiden P, Martin R, Sospedra M (2012) T Lymphocyte priming by neutrophil extracellular traps links innate and adaptive immune responses. *J Immunol*188:3150-3159.
10. Janesh P, Kamp VM, Hoffen EV, Visser E, Tak T, et al. (2012) A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1.*J Clin Invest*122:327–336.
11. Berger-Achituv S, Brinkmann V, Abed UA, Kühn LI, Ben-Ezra J, et al. (2013) A proposed role for neutrophil extracellular traps in cancer immunoediting. *Front Immunol*4: 48.[Crossref]
12. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, et al. (2004) Neutrophil extracelular traps kill bacteria. *Science* 303:1532-1535.
13. Brinkmann V, Zychlinsky A (2012) Neutrophil extracellular traps: is immunity the second function of chromatin? *J Cell Biol*198: 773-783.[Crossref]
14. Rodríguez FM, Novak ITC (2016) Costimulatory molecules CD80 and CD86 colocalized in Neutrophil Extracellular Traps (NETs). *J Immunol Infect Dis*3: 103.
15. Rodríguez FM, Novak ITC (2016) May NETs Contain Costimulatory Molecules. *J ImmunoBiol*1:4.
16. Cross A, Bucknall RC, Cassatella MA, Edwards SW, Moots RJ (2003) Synovial Fluid Neutrophils Transcribe and Express Class II Major Histocompatibility Complex Molecules in Rheumatoid Arthritis. *Arthritis Rheum*48: 2796–2806.
17. Sandilands GP, McCrae J, Hill K, Perry M, Baxter D (2006) Major histocompatibility complex class II (DR) antigen and costimulatory molecules on in vitro and in vivo activated human polymorphonuclear neutrophils. *Immunology* 119: 562–571.
18. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, et al. (1999) The immunogical synapse: a molecular machine controlling T cell activation. *Science*285: 221-227.
19. Bromley SK, Burack WR, Johnson KG, Somersalo K, Sims TN, et al. (2001) The immunological synapse. *Annu Rev Immunol*19: 375-396.[Crossref]
20. Lee KH, Holdorf AD, Dustin ML, Chan AC, Allen PM, et al. (2002) T cell receptor signaling precedes immunological synapse formation. *Science*295: 1539-1542.
21. Matsushima H, Geng S, Lu R, Okamoto T, Yao Y, et al. (2013) Neutrophil differentiation into a unique hybrid population exhibiting dual phenotype and functionality of neutrophils and dendritic cells. *Blood* 121: 1677–1689.
22. Culshaw S, Millington OR, Brewer JM, McInnes IB (2008) Murine neutrophils present Class II restricted antigen. *Immunol Lett*118: 49-54.
23. Ostanin DV, Kurmaeva E, Furr K, Bao R, Hoffman J, et al. (2012) Acquisition of antigen-presenting functions by neutrophils isolated from mice with chronic colitis. *J Immunol*188: 1491–1502.
24. Geng S, Matsushima H, Okamoto T, Yao Y, Lu R, et al.(2013) Emergence, origin, and function of neutrophil-dendritic cell hybrids in experimentally induced inflammatory lesions in mice. *Blood* 121: 1690–1700.
25. Rodríguez FM, Orquera AD, Maturano MR, Infante NS, CarabajalMiotti C, et al. (2016) Human neutrophils in patients with positive serology for Chagas disease. *J Immunol Infect Dis* 3: 101-108.
26. Rodríguez FM, Vargas A, CarabajalMiotti C, González Silva N, Ruiz de Frattari, et al. (2017) Extracellular Traps and costimulatory molecules in leukocytes of patients with positive serology for Chagas disease. *MOJ Immunol*5:00163.
27. Tosello-Boari J, Vesely MCA, Bermejo DA (2012) IL-17RA Signaling Reduces inflammation and mortality during Trypanosoma cruzi infection by recruiting suppressive IL-10-producing neutrophils. *PLoSPathog*8:e1002658.
28. Fridlender Zvi G, Jing Sun, Samuel Kim, Veena Kapoor, Guanjun Cheng, et al. (2009) Polarization of Tumor-Associated Neutrophil (TAN) Phenotype by TGF-ß: "N1" versus "N2" TAN. *Cancer Cell* 16: 183–194.
29. Iking-Konert C, Vogt S, Radsak M, Wagner C, Hänsch GM, et al. (2001) Polymorphonuclear neutrophils in Wegener's granulomatosis acquire characteristics of antigen presenting cells. *Kidney Int*60: 2247-2262.
30. Yasuhiro T, Takahashi H, Kobayashi M,Hanafusa T, Herndon DN, et al.(2004)Three Different Neutrophil Subsets Exhibited in Mice with Different Susceptibilities to Infection by Methicillin-Resistant Staphylococcus aureus. *Immunity* 21: 215–226.
31. Sandilands GP, Ahmed Z, Perry N, Davison M, Lupton A, et al. (2005) Cross-linking of neutrophil CD11b results in rapid cell surface expression of molecules required for antigen presentation and T-cell activation. *Immunology* 114: 354–368.
32. Hacbarth E, Kajdacsy-Balla A (1986) Low density neutrophils in patients with systemic lupus erythematosus, rheumatoid arthritis, and acute rheumatic fever. *Arthritis Rheum*29:1334–1342.
33. Denny MF, Yalavarthi S, Zhao W, Thacker SG, Anderson M, et al (2010) A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. *J. Immunol*184:3284-3297.

34. Carmona-Rivera C, Kaplan MJ (2013) Low-density granulocytes: a distinct class of neutrophils in systemic autoimmunity. *SeminImmunopathol*35: 455-463

35. Abi Abdallah DS, Egan CE, Butcher BA, Denkers EY (2011) Mouse neutrophils are professional antigen-presenting cells programmed to instruct Th1 and Th17 T-cell differentiation. *IntImmunol*23: 317–326.

36. Clemmensen SN, Bohr CT, Rorvig S, Glenthoj A, Mora-Jensen H, et al. (2012) Olfactomedin 4 defines a subset of human neutrophils. *J LeukocBiol*91: 495–500.

37. Cloke T, Munder M, Bergin P, Herath S, Modolell M, et al. (2013) Phenotypic Alteration of Neutrophils in the Blood of HIV Seropositive Patients. *PLoS One*8: 1–7.

38. Welin A, Amirbeagi F, Christenson K, Björkman L, Björnsdottir H, et al. (2013) The Human Neutrophil Subsets Defined by the Presence or Absence of OLFM4 Both Transmigrate into Tissue In Vivo and Give Rise to Distinct NETs In Vitro. *PLoS One*8: 1–13.

39. Hao S, Andersen M, Yu H (2013) Detection of immune suppressive neutrophils in peripheral blood samples of cancer patients. *Am J Blood Res*3: 239-245.

40. Davey MS, Morgan MP, Liuzzi AR, Tyler CJ, Khan MWA, et al. (2014) Microbe-specific unconventional T cells induce human neutrophil differentiation into antigen cross-presenting cells. *J Immunol*193: 3704–3716.

41. Amsalem H, Kwan M, Hazan A, Zhang J, Jones RL, et al. (2014) Identification of a novel neutrophil population: proangiogenic granulocytes in second-trimester human decidua. *J Immunol*193: 3070-3079.

42. Ssemaganda A, Kindinger L, Bergin P, Nielsen L, Mpendo J, et al. (2014) Characterization of neutrophil subsets in healthy human pregnancies. *PLoS One*9:e85696.

43. Fine N, Hassanpour S, Borenstein A, Sima C, Oveisi M, et al. (2016) Distinct Oral Neutrophil Subsets Define Health and Periodontal Disease States. *J Dent Res*95: 931–938.

44. Wang TT, Zhao YL, Peng LS, Chen N, Chen W, et al. (2017) Tumour-activated neutrophils in gastric cancer foster immune suppression and disease progression through GM-CSF-PD-L1 pathway. *Gut*.

45. Zou JM, Qin J, Li YC, Wang Y, Li D, et al. (2017) IL-35 induces N2 phenotype of neutrophils to promote tumor growth. *Oncotarget*8: 33501-33514.[Crossref]