in AAV patients and that they produce less ROS. However, if this is a consequence of the chronic inflammation or an underlying etiological factor remains to evaluate.

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A12
Phagocytosis of apoptotic cells expressing PR3 impaired macrophage anti-inflammatory reprogramming
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Introduction.— Proteinase 3 (PR3) is the major autoantigen which is the target of autoimmunity in granulomatosis with polyangiitis. This serine protease stored mainly in azurophilic granules of neutrophils has large pro-inflammatory described functions. We recently described that PR3 impairs the resolution of inflammation by interfering with the anti-inflammatory reprogramming of macrophages induced by the phagocytosis of apoptotic cells.

Methods.— Stably transfected Rat Basophilic Leukaemia (RBL) cells were used to express PR3 and its catalytically inactive mutant PR3/S203A. These cells, after UV induced apoptosis, were phagocyted by thioglycollate-elicited peritoneal murine macrophages to obtain a post-phagocytic supernatant. Macrophages were next stimulated by LPS to obtain a post-LPS supernatant. NF-κB activation was analysed by 1) Western blot analysis of IkB and 2) by EMSA in Human Mast Cell line-1 (HMC1) stably transfected to express PR3 and its catalytically inactive mutant PR3/S203A. A recombinant lentivirus vector expressing PR3 was also used to express PR3 in these cells.

Results.— Phagocytosis of apoptotic cells expressing PR3 or PR3S203A by thioglycollate-elicited peritoneal murine macrophages induced a post-phagocytic response. As expected, apoptotic controls RBL cells downregulated the secretion of TNFα after LPS stimulation. In the case of phagocytosis of PR3 expressing cells, this “reprogramming” of macrophages was impaired independently of the catalytic activity of PR3. To explore a direct activation of the NF-κB pathway, we used stably transfected or lentivirus-transduced HMC1 to express PR3. We were able to show that the activation of the NF-κB pathway is stronger for PR3 positive cells even if inactive.

Conclusion.— We provide evidence that PR3, a classical pro-inflammatory serine protease, impaired macrophage reprogramming during effectorosis. Moreover, PR3 has the ability to directly activate the NF-κB pathway and this activity appeared to be independent of its serine protease activity.

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A13
ANCA disease patients have defective Treg function exacerbated by expansion of a suppression-resistant effector population
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Introduction.— The development of pathogenic anti-neutrophil cytoplasmic autoantibodies (ANCAs) can result in systemic small vessel vasculitis. However, the breakdown in immune tolerance that results in the induction and persistence of ANCAs is not well understood. We hypothesized that abnormal T cell regulation is central to disease pathogenesis and demonstrate here two separate abnormalities in T cell regulation in ANCA disease patients.

Patients.— Peripheral blood samples were obtained from patients with ANCA-associated vasculitids (n = 63) and healthy controls (n = 19) for flow cytometric analysis of CD4+ T cell populations. Functional T cell studies were performed with FACS sorted CD4+ T cell populations stimulated with anti-CD3/28.

Results.— Our data demonstrate that Treg frequency in the peripheral blood of active disease patients is increased, but Tregs from patients with ANCA disease have decreased suppressive function. Tregs from active disease patients disproportionately utilize a FOXP3 isoform lacking exon 2, which may alter Treg function. Linear regression analysis demonstrates that an increase in protein expression of exon 2-deficient FOXP3 correlates with a diminished suppressive ability (R² = 0.72). Additionally, we identify a CD4+ T cell population (CD127high CD25intermediate) with increased frequency in the periphery of ANCA disease patients compared to healthy controls (53.5% versus 31.03% of CD4+; P < 0.0001). We have determined that CD25+ T cells are resistant to Treg suppression, produce pro-inflammatory cytokines, and are antigen-experienced.

Conclusion.— In sum, ANCA disease is associated with disruption of the suppressive Treg network in the presence of FOXP3 exon 2-deficiency and an increased frequency of a distinct pro-inflammatory effector T cell subset which comprises the majority of peripheral CD4+ T cells.

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A14
Unraveling the identity of FoxP3+ regulatory T cells in GPA-patients
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Introduction.— Human FoxP3+ Th-cells are heterogeneous in function and include suppressive (TReg) and non-suppressive cells that abundantly secrete proinflammatory cytokines. We have previously shown that FoxP3+ Th-cells are increased in GPA-patients as compared to healthy controls (HC). In this group of patients, however, we observed a defective function of TReg and an increase in the Th-17 cells. These observations prompted us to investigate whether increased FoxP3+ Th-cells in GPA-patients as compared to healthy controls (HC). In this group of patients, however, we observed a defective function of TReg and an increase in the Th-17 cells. These observations prompted us to investigate whether increased FoxP3+ Th-cells in GPA-patients are attributed to an increase in the effector FoxP3+ Th-cells.

Methods.— PBMCs were isolated from 46 GPA-patients in remission and 22 matched HC. Expression of CD4, CD45RO, and FoxP3 were determined in the various FoxP3+ Th-cell subsets after stimulation. Functional T cell analysis demonstrates that an increase in protein expression of exon 2-deficient FOXP3 correlates with a diminished suppressive ability (R² = 0.72). Additionally, we identify a CD4+ T cell population (CD127high CD25intermediate) with increased frequency in the periphery of ANCA disease patients compared to healthy controls (53.5% versus 31.03% of CD4+; P < 0.0001). We have determined that CD25+ T cells are resistant to Treg suppression, produce pro-inflammatory cytokines, and are antigen-experienced.

Conclusion.— In sum, ANCA disease is associated with disruption of the suppressive Treg network in the presence of FOXP3 exon 2-deficiency and an increased frequency of a distinct pro-inflammatory effector T cell subset which comprises the majority of peripheral CD4+ T cells.

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and ANCA-positive patients, whereas lower percentages of $\text{AS}^{\text{T}_{\text{reg}}}$ were observed in ANCA-positive patients as compared to ANCA-negative patients and HC. Importantly, a significant increase in the percentage of IL-17$^+$ and IL-21$^+$ cells was seen within the NONTReg from ANCA-positive patients and HC. Importantly, a significant increase in the percentage of ASTReg was observed in ANCA-positive patients as compared to ANCA-negative patients and HC.

**Conclusion.**—Increased FoxP3 expression in Th-cells from GPA-patients is related to an increase in a subset of non-suppressive Th-cells. Increased production of IL-17 and IL-21 cytokines in $\text{NONT}^{\text{T}_{\text{reg}}}$ cells from ANCA-positive patients, in conjunction with a decrease in their suppressive capacity, points towards their effector function in ANCA production.

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Reference


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**A15**

**Performance of the ACR classification criteria as diagnostic criteria for granulomatosis with polyangiitis (GPA) (Wegener’s) in a respiratory referral centre**

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**Introduction.**—As no diagnostic criteria for granulomatosis with polyangiitis (GPA) exist, the ACR classification criteria have been used as surrogate for diagnosis. They have a 88% sensitivity (Se) and 92% specificity (Sp). In spite of the high frequency of respiratory manifestations of GPA, and the presence of two items of the respiratory system in the ACR criteria, their evaluation as diagnosis surrogate in a respiratory centre has not, to our knowledge, been reported.

**Patients.**—The ACR classification criteria were applied to consecutive, prospective (in- and outpatients) subjects with suspicion of GPA attending a nationwide respiratory referral centre. Diagnosis was based on clinical, serological and histopathological data. Sample size estimation was calculated for Se of 50–90% and Sp of 75–90% [1]. Se, Sp, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios (PLR and NLR, respectively) and area under the curve (AUC) were estimated.

**Results.**—Ninety-three patients with clinical suspicion of GPA were included. Thirteen (14%) had the disease. With 2/4 ACR criteria Se was 31%, Sp 56%, PPV 10%, NPV 83%, PLR 0.69, NLR 1.23 and AUC 0.5.

**Discussion.**—ACR classification criteria for GPA derived from data of cohorts in where patients had already an established diagnosis. Their performance as surrogate for diagnosis is questionable. Most validation studies have been done in rheumatological or nephrological units. Determining their value as diagnostic surrogates in defined settings, with GPA being a multisystemic disease, is useful. We have explored the performance respiratory diseases referral centre prospectively, by including all consecutive patients with GPA suspicion.

**Conclusion.**—In our setting, ACR classification criteria have poor performance when used as diagnostic surrogates. This highlights the relevance of the ongoing DCVAS project for classification and diagnostic criteria of the vasculitides.

Reference


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**Introduction.**—The classification of the ANCA-associated vasculitides (AAV) (GPA, MPA, EGPA) and PAN remains controversial. The ACR classification criteria whilst validated do not include MPA, whilst the Chapel Hill Consensus Conference (CHCC) definitions promulgated in 1994 were not intended to be used for classification. In 2005, an international consensus group produced the European Medicines Agency (EMA) algorithm for classification of AAV [1]. It combines the ACR and CHCC definitions. In 2012 the CHCC definitions were revised to include updated information in particular the role of ANCA [2]. The EMA algorithm was validated using a cohort of 99 patients with systemic vasculitis from a single-centre [1].

The aim of this study was to reclassify the same 99 patients using the CHCC 2012 definitions to determine whether the new definitions changed the classification of patients with AAV.

**Methods.**—The original dataset from the 99 patients used in the validation of the algorithm was used to reclassify the patients. The reclassification was performed by an independent observer (RA) who was not involved in the original study and was blinded to the diagnosis/previous classification of the patients.

**Results.**—Using the 2012, definitions 59/99 patients were classified with GPA, 22 MPA and 18 EGPA. There were no unclassified patients. Using the 1994 definitions there were 59 GPA, 22 MPA and 18 EGPA. However, one patient changed from MPA to GPA and one GPA to MPA. The kappa statistic was 0.96. The patient who changed from MPA to GPA reflected a difference in the interpretation of the significance of renal involvement. The patient who switched from GPA to MPA reflected a change in the interpretation of infiltrates on chest X-ray.

**Discussion.**—The revision of the CHCC definitions makes no difference to the performance of the EMA algorithm for the classification of AAV.

**Conclusion.**—The EMA algorithm for the classification of AAV is a valid algorithm for classifying patients with AAV using the CHCC 2012 definitions.

Reference


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