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 be evaluated.

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A12
Phagocytosis of apoptotic cells expressing PR3 impaired macrophage anti-inflammatory reprogramming
A. Millet, J. Mocck, M. Pedzeroli-Ribeil, F. Cormier, V. Baud, V. Wilko-Sarsat
Cochin Institute, Paris, France

Introduction.—Proteasease 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 phagocytosed by thiglycollate-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 thiglycollate-elicited peritoneal 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
UNC Chapel Hill, Chapel Hill, USA

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 ANCs 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 vasculitis (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 (CD127-high CD25-intermediate) 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
W.H. Abdullaah1, C.A. Stegemann2, M.G. Huitema1, P.C. Limburg3, A. Rutgers4, P. Heeringa4, C.G.M. Kallenberg1
1. Department of Rheumatology and Clinical Immunology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
2. Department of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
3. Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
4. Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

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 compared to healthy controls (HC) 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 (CD127-high CD25-intermediate) 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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