Patients.— We identified 21 Hispanics and 25 Caucasians treated for AAV at Rush University in Chicago, IL from January 2006 to December 2011. Ethnicity was determined by self-report. Patient demographics, laboratory data, Birmingham Vasculitis Activity Score (BVAS), and Vasculitis Damage Index (VDI) were analyzed. Student’s t-test and chi-square tests were employed, \( P = 0.05 \) was considered significant.

Results.— Of the 46 patients, 27 had GPA, 12 had MPA, seven had CSS. There was no difference in the median age at diagnosis, time to diagnosis, or in the gender distribution between Caucasians and Hispanics. Hispanics had a higher mean BVAS at presentation (16.75 \pm 7.7 versus 12.4 \pm 6.7, \( P = 0.03 \)), a higher mean VDI at presentation (2.9 \pm 1.5 versus 1.9 \pm 1.2, \( P = 0.03 \)) and a cumulative mean VDI (3.9 \pm 1.7 versus 2.5 \pm 1.9, \( P = 0.01 \)) compared to Caucasians. The majority of Hispanics presented with renal involvement (89% of Hispanic versus 56% of Caucasians, \( P = 0.06 \)) however the difference did not reach statistical significance. Seventy percent of Hispanics had acute renal failure (Mean highest creatinine = 4.01 \pm 3.01 mg/dL) of whom half required dialysis, versus 29% of Caucasians (Mean highest creatinine = 1.98 \pm 1.67 mg/dL, \( P = 0.05 \)) and only two patient’s requiring dialysis. Two Hispanic patients (11%) died shortly after presentation. There were no deaths among Caucasians.

Conclusion.— Hispanics with AAV presented with more systemic and severe disease with higher damage index as compared to Caucasians. Whether these differences are due to genetic, socio-economic or healthcare access disparities is yet to be studied.

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A42

Genetic regulation of CD177 – A receptor presenting anti-neutrophil cytoplasmic antigen proteinase 3

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Introduction.— ANCA activate neutrophils leading to necrotizing vasculitis and crescentic glomerulonephritis (CGN). CD177 (Neutrophil anti-A receptor B1) presents the major ANCA antigen proteinase 3 (PR3) on the neutrophil membrane (mPR3) yielding CD177pos/mPR3low and CD177pos/mPR3high subsets. The percentage of CD177pos/mPR3high neutrophils confers risk for ANCA vasculitis, is associated with worse clinical course and a stronger activation response to PR3-ANCA in vitro. The mechanisms that control CD177 protein expression are incompletely understood.

Methods.— We used cell sorting, isolated DNA and mRNA, performed PCR, Southern blot analysis, DNA and cDNA sequencing as well as Affymetrix chip analysis.

Results.— We detected CD177 mRNA in CD177pos/mPR3high neutrophils, but neither full-length nor truncated mRNA in CD177neg/mPR3low cells. By Southern blot analysis of the CD177 gene we excluded genomic recombination with a neighboring pseudogene and copy number variations of the CD177 locus to be causes of different CD177 gene expression. Structural DNA aberrations and single nucleotide variations were excluded for both subsets by genome-wide human SNP array 6.0 from Affymetrix™ suggesting no differences in the DNA sequences between both subsets. DNA sequences in exon-coding region and in exon-intron transitions of the CD177 gene did also not differ between both subsets. Haplotyp analysis in 13 parent-offspring trios identified 12 informative heterozygous SNPs revealing monoallelic CD177 expression in 11 of 13 trios with no informative SNPs in the remaining two trios. We identified the parental-allelic origin in eight of 11 trios (2 maternal, 6 paternal). Monoallelic expression was non-synonymous in nine of 11 children affecting the primary CD177 protein structure.

Discussion.— The findings provide novel insight into CD177 gene regulation and could facilitate studying CD177-related mechanisms in ANCA-associated vasculitis and additional neutrophil-mediated diseases.

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A43

ANCA disease patients with increased expression of autoantigen genes produce an alternative PR3 transcript and synthesize autoantigen proteins


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Introduction.— Generally, mature neutrophils minimally synthesize RNA and protein. However, peripheral neutrophils from ANCA patients are transcribing genes, including the autoantigen PR3. Since mature neutrophils already contain stores of PR3 protein we asked two questions regarding increased PR3 expression in ANCA patients: Are there alternative PR3 transcripts produced in ANCA patients? Is PR3 message translated in mature neutrophils?

There are two annotated transcripts from the PR3 gene. One transcript contains all five exons of the PR3 gene; the other encodes a form originally described as myeloblastin (MBN). We explored the frequency of both PR3 and MBN transcripts in our patient population.

Methods.— Total leukocyte mRNA was isolated from patients with ANCA disease (BVAS range 0–26) and control subjects and screened for PR3 and MBN using transcript specific primers. Total PR3 transcript levels were monitored by qPCR. Potential translation of these transcripts was studied in cell lines and patient neutrophils.

Results.— Twenty-eight percent of MPO-ANCA samples (n = 36) and 20% of the PR3-ANCA patient samples (n = 55) were positive for the MBN specific transcript, while all healthy subjects (n = 18) were negative. Fifty-one percent of ANCA patients with a qPCR value for total PR3 expression above 120 are positive for MBN compared to 0% of patients with a qPCR value below 120. Expression of MBN is not due to an influx of progenitors into the periphery. Samples from patients with “left-shift” were negative for the MBN transcript. The exclusive transcription of MBN in MCF-7 cell lines indicates that this gene can be translated. Neutrophils isolated from patients with ANCA vasculitis synthesize both PR3 and MPO In vitro.

Discussion.— ANCA patients actively synthesize an uncommon PR3 transcript. Also, mature neutrophils synthesize their autoantigen.

Conclusion.— The presence of MBN transcript and evidence of new ANCA antigen protein synthesis further support perturbed neutrophil physiology that may contribute to increased neutrophil activation and antigen exposure.

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