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Abstracts

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B Cell Phenotypes of ANCA Patients Differ from Healthy Controls and Correlate with Disease Activity

D.O. Bunch, H. Chin, S.L. Hogan, P. Sullivan, S.H. Clarke, R.J. Falk, P.H. Nachman

University of Carolina, Medicine/Nephrology & Kidney Center, Chapel Hill, USA

The regulation of the ANCA autoimmune response is poorly understood. To investigate B cell regulation, we performed flow cytometry analysis of lymphocyte samples from 19 patients with ANCA glomerulonephritis of varying levels of disease activity, 14 healthy controls, and 10 patients with systemic lupus erythematosus (SLE) as disease controls. Patients were classified as being in remission or having active disease. Lymphocytes were stained with antibodies to IgM, IgD, IgG, CD19, CD27, and CD38, in addition to biotinylated myeloperoxidase (MPO) or proteinase 3 (PR3) detected with fluorescent-labeled Streptavidin. B cells were gated based on CD19+ staining. CD38 and IgD were used together to categorize B cells from naïve to memory B cells (Bm1–Bm5). Patients with MPO-ANCA and PR3-ANCA active disease (but not patients in remission) exhibit an increase in activated naïve Bm2 cells ($p = 0.01$) and decreases in memory B cells (both early Bm5 and Bm5 cells), germinal center founder cells, and naïve Bm1 cells ($p = 0.02$). No differences were noted between patients with MPO and PR3 ANCA. Furthermore, ANCA-specific B cells were detected in 5 of 19 patients. Using IgD and CD27, we classified B cells as naïve cells, switched and non-switched memory B cells. Active ANCA patients show a significant loss of non-switched memory cells ($p = 0.02$) and a more variable loss of switched memory cells. In one patient with de novo active vasculitis, the ‘loss’ of memory B cells preceded the initiation of immunosuppressive treatment. PR3-ANCA patients in remission exhibited a pattern similar to that of healthy controls, whereas 2 of 4 MPO ANCA patients in remission had loss of switched and non-switched memory cells. Our data suggest an altered B cell phenotype distribution in patients with active ANCA vasculitis. The study of B cell subpopulations may provide insight as to the regulation of the ANCA autoimmune response.

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CD8+CD28– T-cell Lymphocytes in Patients with Wegener’s Granulomatosis: Cytotoxic Effector Cells, Progressing towards Senescence during the Course of the Disease

C. Iking-Konert, T. Vogl, B. Ostendorf, C. Wagner, B. Prior, M. Schneider, K. Andrassy, G.M. Hänsch

Rheumazentrum Düsseldorf, University of Düsseldorf, Düsseldorf, Germany

Objectives: The etiology and pathogenesis of Wegener’s granulomatosis (WG) are still elusive; there is, however, strong evidence for the activation of the T-lymphocytes compartment. In that context, we studied lineage-typical and function-associated surface receptors of T-lymphocytes of patients with WG.

Methods: In patients with WG ($n = 56$) the expression of CD28, CD11b, CD57, CD27, CD62L, CCR7 on CD8+ T-cells was measured by FACS analysis. In parallel T-cell lines of healthy donors and patients where studied in vitro. For comparison cells of healthy, age-matched individuals (NHD) were studied.

Results: The most intriguing finding was the reduced expression of the co-stimulatory molecules CD27 and CD28, particularly of the CD8 positive T-cells. In patients with long-lasting, severe disease (>5 yrs., systemic disease with multiple relapses) up to 75% of CD8+ T-cells were negative for CD28 (mean $\pm$ SD 52.5 ± 24.0 versus 35.5 ± 17.5 for NHD, and 28.9 ± 14.9 for patients with acute bacterial infections). The majority of the CD8+CD28– cells also expressed CD11b, CD57, while CCR7 and CD62L were lost. CD11b and CD57 were co-expressed on about 50% of the CD8+CD28– cells. In vitro experiments revealed that up-regulation of CD11b coincided with the expression of perforin and granzyme B. On cell lines expanded in vitro, CD57 expression was seen concomitantly with a shortening of the telomeres, synthesis of gamma interferon and the failure of the cells to proliferate in response to mitogens.

Conclusion: In summary, in patients with WG, CD8+CD28– T-cells are found. These represent cytotoxic T-cells, which progress towards senescence during the course of the disease. In conclusion, our data provide evidence that lymphocytes within the CD8+ compartment are activated in patients with primary vasculitis.

Persistent Expansion of T-helper-2 Effector Memory T-cells in Wegener's Granulomatosis

W.H. Abdulahad, Y.M. van der Geld, C.A. Stegeman, C.G.M. Kallenberg

University Medical Center Groningen, Clinical Immunology, Groningen, Netherlands

Objective: In order to test the hypothesis that the presence of Wegener's granulomatosis (WG) also in remission is associated with an ongoing immune effector response, we examined the distribution of peripheral naïve and memory T-lymphocytes and analyzed the function-related phenotypes of the memory T-cell population.

Methods: Peripheral blood mononuclear cells (PBMCs) were freshly isolated from WG-patients in remission (R-WG, $n = 40$), active WG-patients (A-WG, $n = 17$), and age- and sex-matched healthy controls (HC, $n = 21$). The cell surface expression of CD4, CD8, CD45RO, CCR7, IL-18, and ST2L were determined by four-color flow cytometric analysis. The expression of IL-18 and ST2L were examined to clarify the presence and distribution of Type1- and Type2 T-cells, respectively.

Results: In R-WG, the CD4+CD45RO+CCR7- effector memory T-cell subpopulation (TEM) was increased, whereas the CD4+CD45RO-CCR7+ naïve T-cell population (TNaive) was decreased as compared to HC. No such disturbances in T-cell distribution were found in A-WG. The percentage of CD4+CD45RO+CCR7+ central memory T-cells (TCM) did not differ between R-WG and A-WG and HC, nor did the distribution of naïve and memory CD8+ T-cells. In contrast to HC, the percentage of CD4+TNaive cells in R-WG correlated negatively with age, while CD4+TEM cells showed a positive correlation. In R-WG a skewing towards Type2 T-cells was observed in CD4+TEM cells, while CD4+TCM cells were skewed towards Type1 T-cells.

Conclusion: Peripheral blood homeostasis of CD4+T-cells is disturbed in R-WG with the persistent expansion of Type2 CD4+TEM cells. These cells might be responsible for the relapsing course of WG and may constitute a target for therapy.

Persistent T-Cell Activation and Clinical Correlations in Patients with ANCA-Associated Systemic Vasculitis

A.-I. Kälisch, S. Marinaki, P. Grimminger, A. Breedijk, R. Birck, W.H. Schmitt, F.J. van der Woude, B.A. Yard

University of Heidelberg, Klinikum Mannheim, Fifth Medical Department, Mannheim, Germany

Although in ANCA-associated systemic vasculitis (AASV) patients, activation of T-cells have been described, persistence of these alterations in peripheral blood T-cells is not well characterized. This study was conducted to define persistent T-cell activation (PTA) in AASV patients and to assess if this correlates with clinical parameters. Moreover, we investigated whether T-cells from patients with persistent

T-cell activation were functionally different. To this end, the expression of CD4, CD45RO, CD25, CD26, CD28, CCR7 and HLA-DR was examined longitudinally in 38 consecutive AASV patients. Clinical parameters were compared by univariate and multivariate analysis and Kaplan meier curves for relapse free survival were calculated. Additionally, intracellular cytokines and FOXP3 mRNA were measured by FACS-analysis and real time PCR respectively. PTA could be defined as either of two activation phenotypes, i.e. a low percentage of CD4+CD45RO- T-cells or a high percentage of CD25 in the naïve CD4+population ($n = 26$), since only these phenotypes were stable over time. It was not associated with disease activity, but was significantly more often found in patients with major organ involvement. In addition, PTA was associated with age and cumulative cyclophosphamide dose but not with relapse free survival. Patients with a low percentage of CD4+CD45RO- T-cells were significantly more often diagnosed as microscopic polyangiitis. No evidence for a prevalence for Th1 or Th2 could be demonstrated in PTA. Similarly, FOXP3 expression was not different in patients with or without PTA.

Upregulated NKG2D Expression on CD4+CD28- T-cells in Wegener's Granulomatosis

A. Müller, D. Capraru, K. Holl-Ulrich, E. Csernok, J. Voswinkel, W.L. Gross, P. Lamprecht

University of Lübeck, Department of Rheumatology, Lübeck, Germany

Background: Failure to adequately control T-cell activity may result in unwanted sequelae such as granuloma formation and autoimmune vasculitis, both of which are hallmarks of Wegener's granulomatosis (WG). Recently it has been suggested that up-regulation of the non-constitutively expressed co-stimulatory receptor NKG2D on CD4+ T-cells might facilitate autoantigen-recognition in stressed tissues expressing the NKG2D ligand MIC (non-classical MHC-class I-like chain). To characterize potential mechanisms of uncontrolled T-cell activation in WG, we analyzed NKG2D expression on peripheral blood T-cells and its ligand MIC in granulomatous lesions.

Methods: Flow-cytometric analysis of NKG2D expression and other markers on circulating T-cells in WG and healthy controls ($n = 20$ /group). Immunohistochemistry studies were performed to study MIC expression in granulomatous lesions ($n = 5$).

Results: Expression of the co-stimulatory molecule CD28 was significantly reduced on CD4+ T-cells ($87.2 \pm 3.7\%$, mean $\pm$ SEM) and CD8+ T-cells ($54.5 \pm 4.9\%$) in WG compared to healthy controls ($98.4 \pm 0.6\%$, $69.8 \pm 4.4\%$, $p < 0.01$, $p < 0.05$, respectively). NKG2D was constitutively expressed on CD8+ T-cells, but not on CD4+ T-cells in healthy individuals. In contrast, NKG2D expression was strongly up-regulated and preferentially expressed on the majority of the expanded CD28-CD27- T-cell fraction within the CD4+ T-cell population in WG. Blocking of NKG2D reduced IFN- γ secretion of PR3-specific CD4+ T-cells. Immunohistochemical stainings disclosed MIC, NKG2D, and autoantigen (proteinase 3) expression in granulomatous lesions.

Conclusions: Circulating T-cells lacking co-stimulatory CD28 and CD27 expression are expanded in WG. The phenotype displayed

by the expanded CD4+CD28–CD27–NKG2D+ T-cell fraction is that of late differentiated, senescent effector memory T-cells. In WG anomalous NKG2D expression might facilitate autoantigen recognition in granulomatous lesions.

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Regulation of Lymphocyte Responses in MPO-ANCA Vasculitis

K. Chavele, D. Shukla, T. Ketepe-Arachi, D. Fuchs, C.D. Pusey, A.D. Salama

Imperial College London, Hammersmith Hospital, Renal Section, Division of Medicine, London, United Kingdom

T lymphocytes have been implicated in the pathogenesis of ANCA vasculitis, as they are found in the glomeruli of affected patients, provide help for B cell Ig production and have been shown to proliferate in vitro to the known autoantigens. Moreover, since patients may have high ANCA titres and no evidence of clinical disease, it suggests that the autoimmune disease is not controlled uniquely at the antibody level. Thus we investigated regulation of MPO-specific T cells in patients with MPO-ANCA vasculitis ($n = 23$) and healthy controls. In patients with acute disease (BVAS > 6) MPO-specific IFN- γ and IL-4-producing T cell frequencies were elevated and these diminished to levels found in healthy controls during disease remission (mean BVAS < 1). The low MPO-specific T cell frequencies during disease convalescence were not due to the effect of CD25+ regulatory cells, as frequencies did not change following Treg depletion and foxp3 levels were not elevated by real time PCR. Neither were they due to T cell anergy, as they were not reversed by the addition of exogenous IL-2. We investigated other Methods of T cell regulation including activation of the 2,3 indoleamine dioxygenase (IDO) pathway. Interestingly, we found elevated serum levels of kynurenine and low levels of tryptophan (adjusted for creatinine) in the patients compared to controls ($p = 0.01$). However, in vitro blockade of peripheral leucocyte IDO, by the antagonist 1-methyltryptophan, did not augment T cell responses to MPO, suggesting that localised production may be more important and result in overspill into the circulation.

Conclusions: T cell frequencies to MPO are elevated in acute disease, and are at control levels during disease convalescence. This may explain the low relapse rates in this cohort of patients. Regulation appears to be deletional rather than due to active suppression and may in part relate to low tryptophan and high kynurenine levels, promoting T cell unresponsiveness and apoptosis.

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T Cell Activation Profiles in Kawasaki Disease

P.A. Brogan, V. Shah, L. Clarke, N. Klein, M.J. Dillon

Institute of Child Health, Department of Rheumatology, London, United Kingdom

Introduction: Superantigens cause sequential activation then expansion and ultimately deletion of T cells expressing specific V-beta

sub-units of the T cell receptor. Studies of T cell V-beta repertoires in children with Kawasaki disease (KD) have inconsistently revealed expansions and deletions of V-beta 2 and V-beta 8 T cells, although studies examining V-beta specific T cell activation in the absence of expansions or deletions have so far been lacking.

Aims and Methods: In addition to examining T cell V-beta repertoires by FACS analysis, this study examined V-beta restricted T cell activation (V-beta-restricted CD69 expression) in peripheral blood from 16 children with KD, as compared to 25 healthy controls, and 25 disease controls.

Results: Comparison of the groups overall revealed increased expression of CD4 T cells expressing V-beta 2. Detailed examination of the V-beta repertoires from individual KD patients revealed that 7/16 patients had other major V-beta expansions and/or deletions throughout the CD4 and CD8 V-beta repertoire, involving (in order of frequency) V-beta 5.1, V-beta 2, V-beta 3, V-beta 1, and V-beta 12. Further examination of 10 of the KD patients additionally revealed evidence of V-beta restricted CD4 and CD8 activation as determined by a log shift in CD69 median fluorescence index (MFI) of specific V-beta families in 5 of the 10 KD patients. V-beta restricted activation was observed in CD4 V-beta 3, 8 and 12; and CD8 V-beta 1, 2, 3, 5.1, 8 and 12. This occurred in 4 children who did not demonstrate major V-beta expansions or deletions.

Conclusions: 11 of the 16 KD patients had evidence of V-beta percentage skewing and/or V-beta restricted T cell activation. We suggest that the notably inconsistent observation of V-beta repertoire skewing in KD could partly be explained by the fact that some children exhibit V-beta restricted activation without expansion or deletion of specific V-beta families, and that absence of percentage skewing of the peripheral blood T cell V-beta repertoire does not preclude a superantigenic-mediated trigger for KD.

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No Role for the CD25high Regulatory T Cell (T Reg) in the Maintenance of Remission in Wegener's Granulomatosis

C. Day, J.S. Steele, C.O.S. Savage

Medical School, University of Birmingham, Renal Immunobiology, Birmingham, United Kingdom

Wegener's granulomatosis (WG) is associated with proteinase 3 (PR3)-specific anti-neutrophil cytoplasm antibody (ANCA) presence and with a high degree of T-helper-1 cell (TH1, IFN- α producing) activation in active disease. Previous investigators have shown the presence of PR3-specific T cells by proliferation. Recent evidence has suggested a role for CD25+ T regs in the regulation of autoimmune disease. We set out to examine the control of relapse in WG. Fourteen patients with WG, eleven in remission and three with more active disease, and eleven healthy controls were studied. All but one of the patients was taking immunosuppressive therapy and thus the sensitive dendritic cell ELISpot was employed. Depletion of CD25-high cells was carried out using Dynal beads. Only two patients, and no controls, showed the presence of IFN- α producing PR3-specific T cells and their presence did not correlate with disease activity. Depletion of CD25high cells did not cause a significant increase in the frequency of such cells detected in either patients in remission

or controls. It would appear that CD25^{high} T regs do not maintain remission in WG by suppressing the activity of PR3-specific TH1 cells. However, alternatives are also possible. Our studies show an increased fraction of activated T cells expressing CD25 in patients with active vasculitis that remains above control levels even in remission. The presence of these cells could complicate the interpretation of the data if antigen-responsive T cells were removed along with T regs. Alternatively, the peripherally produced Tr1 cell, that is thought to be less consistently CD25^{high}, may have an important role to play in the prevention of relapse in WG. It may be that changes in PR3 itself occur in WG. Changes in glycosylation profiles have been shown to occur in other autoimmune diseases and may be important in creating neo-epitopes. A more controversial explanation might be that PR3-specific T cells have no role to play in WG.

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Complementary PR3 Peptide Activates CD4⁺ T Cells from Patients with PR3-ANCA-Disease

Y. Jiajin, B.M. Pressler, W.F. Pendergraft, C.M. Nester, J.C. Jennette, R.J. Falk, G.A. Preston

University of North Carolina, Medicine, Chapel Hill, USA

We hypothesized that within the repertoire of T-lymphocytes of a proteinase 3-antineutrophil cytoplasmic autoantibodies (PR3-ANCA) patient there exists a subset that are responsive to complementary PR3. The rationale for this hypothesis is based on the discovery that PR3-ANCA patients harbor antibodies (IgG) against complementary PR3, implying that a cognate population of T cells may exist. Peripheral blood mononuclear cells from PR3-ANCA patients ($n = 14$) and healthy donors ($n = 13$) were stimulated with a complementary PR3138–169 peptide (cPR3138–169), a sense PR3138–169 peptide (PR3138–169), heat-inactivated PR3, or recall antigen cocktail. Proliferation (cell division index, CDI) of lymphocytes was determined by flow cytometry as a decrease of CFDA fluorescence intensity. Lymphocytes were sorted by CD3, CD4, and CD8 surface marker staining. T cells from 43% of PR3-ANCA patients proliferated in response to cPR3138–169, compared to 0% of healthy controls ($p = 0.04$). Quantitation of proliferative T-cells responsive to cPR3138–169 gave a CDI of 9.0 ± 10.9 for PR3-ANCA patients, while the CDI for healthy donors was 1.5 ± 1.1 , a statistically significant difference ($p = 0.03$). The sense PR3138–169 did not induce proliferation in either group (CDI: 1.8 ± 1.8 ; 1.1 ± 0.5 , $P = 0.23$). We found that PR3 stimulated a positive response in both groups (36% vs. 8%, $p = 0.16$), however this response was more robust in PR3-ANCA patients (CDI: 6.4 ± 7.2 ; 1.8 ± 1.0 , $p = 0.04$). Recall antigen cocktail induced proliferation in both groups (CDI: 16.9 ± 17.5 ; 17.0 ± 24.4). The ELISpot and flow cytometry Methods are now being used to determine cytokine responses. cPR3138–169-induced T-cell proliferation results in production of IFN- γ by CD4⁺ cells, but not CD8⁺ cells. The presence of reactive T cells implies that the inciting antigen in ANCA disease may be a protein complementary to PR3. The data extend and support the theory of autoantigen complementarity and its role in development of ANCA-disease.

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Endogenous MPO and PR3 may Provide Source of ANCA Antigens during Monocyte Differentiation into Dendritic Cells

P. Hurtado

Royal Adelaide Hospital, Renal Unit, Adelaide, South Australia, Australia

Objective: To study the fate of MPO and PR3 as monocytes differentiate into monocyte-derived dendritic cells (MDDC).

Methods: Monocytes were isolated from healthy donor buffy coats with immunomagnetic beads and cultured with GM-CSF and IL-4 for 5–7 days to induce differentiation into MDDC. Intracellular expression and distribution of MPO, PR3, HLA-class II and HLA-DM were analysed by FACS and confocal fluorescence microscopy. MPO and PR3 mRNA were quantified by RT-PCR. MPO in cell culture supernatant was measured by ELISA.

Results: During differentiation into MDDC, the intracellular content of MPO and PR3 gradually decreased to almost undetectable levels by day 7 of culture. This was shown by FACS and confirmed by confocal microscopy. The release of MPO was maximal in the first 12hr of culture, followed by progressive decline in release over successive days. There was substantial reduction of MPO mRNA over time whereas PR3 mRNA was absent throughout. Even though day 5 MDDC contained minimal amounts of MPO and PR3, it was evident by confocal microscopy that significant proportions of these antigens co-localised with HLA-class II and HLA-DM containing lysosomal compartments. Furthermore, inhibition of lysosomal function by leupeptin and ammonium chloride halted the intracellular degradation of MPO and PR3.

Conclusions: Intracellular MPO and PR3 are purged during monocyte differentiation into MDDC. Besides extracellular release and shutting down of mRNA transcription, we provide evidence that lysosomal degradation contributes to this process. Lysosomal degradation of endogenous MPO and PR3 during monocyte differentiation into MDDC is potentially important because peptides derived from these antigens may be presented by MHC-class II molecules on DCs. This may constitute a normal physiological process by which peripheral tolerance to MPO and PR3 is maintained, but under different circumstances may trigger autoimmunity.

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Evidence for Autoantigen Complementarity in Anti-glomerular Basement Disease

B.M. Pressler, J.A. Astern, W.F. Pendergraft, J.C. Jennette, G.A. Preston, R.J. Falk

University of North Carolina, Medicine/Nephrology and Hypertension, Chapel Hill, USA

We hypothesized that, in addition to anti-collagen autoantibodies, patients with anti-glomerular basement membrane (anti-GBM) disease harbor antibodies reactive against a protein complementary in amino acid sequence to the known collagen epitope, $\alpha 3(\text{IV})\text{NC1}$. This hypothesis is based on principles delineated in the theory of

autoantigen complementarity, which proposes that autoimmune diseases are triggered by an immune response to proteins complementary to autoantigens. To test whether a protein complementary to alpha3(IV)NC1 is involved in the immunogenesis of anti-GBM disease, two approaches were taken. Firstly, a 20-amino acid peptide was synthesized by transcription of the mRNA sequence transcribed from the non-coding (i.e. antisense) DNA strand of the epitope region of the alpha3(IV)NC1 gene; a stop codon corresponding to a serine residue in the epitope was replaced with glycine, and 25 intervening amino acids were eliminated to bring a distal antigenic residue into closer proximity. Secondly, a 65-amino acid recombinant protein—comprised of the entire epitope region with all of the intervening amino acids—was produced in and purified from human embryonic kidney cells. Positive seroreactivity by ELISA was defined as an OD value 2 standard deviations above the mean of the normal controls. Of anti-GBM patients, 7/15 (47%) were positive against synthetic peptide and 6/27 (22%) were positive against recombinant protein; of 16 patients tested against both, 6 (38%) were positive against synthetic peptide, and 4 of these 6 were also positive against recombinant protein. It appears that anti-complementary peptide reactivity is not due to cross-reactivity of the autoantibody, as purified anti-alpha3(IV)NC1 antibodies from a patient's total IgG were not reactive, but flow-through retained seroreactivity. These preliminary data imply that autoantigen complementarity may play a role in anti-GBM disease, and that this theory may not be a PR3-ANCA-specific phenomenon.

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Evidence that Complementary Proteins May Play a Role in the Immunogenesis of MPO-ANCA

J.A. Astern, B.M. Pressler, W.F. Pendergraft III, C.M. Trent, J.C. Jennette, G.A. Preston, R.J. Falk

University of North Carolina, Medicine/Nephrology and Hypertension, Chapel Hill, USA

The Theory of Autoantigen Complementarity proposes that autoimmune diseases are initiated by an immune response against a protein complementary in amino acid sequence to the autoantigen. A subsequent anti-idiotypic response generates an antibody that reacts with the autoantigen. Our research group previously implicated complementary proteins in the pathogenesis of PR3-ANCA disease; we hypothesize that patients with MPO-ANCA also harbor antibodies specific to a protein complementary in sequence to MPO. To test our hypothesis, we generated a complementary recombinant protein fragment coded by the 5' to 3' antisense sequence of the MPO gene corresponding to sense amino acids 420–697 [cMPO(420–697)]. Studies by our group and others have implicated immunodominant regions in the carboxy-terminus of the MPO protein, thus we designed the cMPO(420–697) fragment to correlate with these broadly defined regions. We tested 19 patient serum samples and 26 healthy control serum samples for reactivity to hcMPO(420–697) by ELISA. Positive seroreactivity was defined as an OD value 2 standard deviations above the mean of the healthy controls. 6/19 (32%) MPO-ANCA patient samples were positive against cMPO(420–697) (p value = 0.03, Fisher's Exact Test). Two of the positive samples were different blood draws (one year apart) from the same patient. This data suggests

that autoantigen complementarity may explain the genesis of MPO-ANCA, and this theory is not a PR3-ANCA-specific phenomenon.

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A Pathogenic Role for Alternative Pathway Complement Activation in Anti-MPO Induced Necrotizing and Crescentic Glomerulonephritis

H. Xiao, P. Heeringa, A. Schreiber, R.J. Falk, J.C. Jennette

University of North Carolina, Pathology, Chapel Hill, USA

Complement activation is involved in the pathogenesis of inflammatory disease not only through classical pathway activation by immune complexes but also alternative pathway activation that does not require immune complexes. We investigated the role of complement in a mouse model of necrotizing and crescentic glomerulonephritis induced by anti-MPO antibodies. C57BL/6j (B6) mice ($n = 3$) and Rag2 $^{-/-}$ mice ($n = 7$) were depleted of complement with 30 μ g/0.5 ml PBS of Cobra venom factor (CVF) i.p. Control B6 ($n = 4$) or Rag2 $^{-/-}$ ($n = 7$) mice received 0.5 ml PBS. 4 hours after CVF or PBS, B6 mice received anti-MPO IgG and Rag2 $^{-/-}$ mice received 5×10^7 anti-MPO splenocytes. All complement depleted mice had normal urine (B6) or mild abnormalities (Rag2 $^{-/-}$), and had no glomerular necrosis or crescents. All B6 and Rag2 $^{-/-}$ mice that did not receive CVF had proteinuria, hematuria and leukocyturia; and all had glomerular necrosis (B6 5%, Rag2 $^{-/-}$ 36%) and crescents (B6 11%, Rag2 $^{-/-}$ 37%). To identify the involved pathway, anti-MPO IgG was injected into WT B6 mice ($n = 5$), C4 $^{-/-}$ mice ($n = 6$), Factor B $^{-/-}$ mice ($n = 8$) or C5 $^{-/-}$ mice ($n = 3$). All WT B6 mice developed glomerular necrosis (9%) and crescents (20%), and all C4 $^{-/-}$ mice developed glomerular necrosis (9%) and crescents (18%). None of the Factor B $^{-/-}$ mice or C5 $^{-/-}$ mice developed glomerulonephritis. All groups of mice had similar serum anti-MPO IgG, and all had a paucity/absence of IgG, IgA, IgM and C3 in glomeruli. These studies demonstrate that blockade of the alternative complement pathway but not the classical pathway prevents anti-MPO glomerulonephritis. We hypothesize that neutrophils activated by ANCA initiate an innate inflammatory amplification loop that involves both generation of C3a and C5a by neutrophils and stimulation of neutrophils through C3aR and C5R.

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Rats Immunized with Chimeric Human/mouse PR3 Proteins Produce Autoantibodies to Rat Granulocytes

Y.M. van der Geld, T. Hellmark, C.G.M. Kallenberg

UMCG, Clinical Immunology, Groningen, The Netherlands

1 University medical center Groningen. Dept. Clinical immunology, Groningen, The Netherlands, 2 Lund University hospital, Dept. of Nephrology, Lund, Sweden. PR3-ANCA have been suggested to play a

direct role in the pathogenesis of PR3-ANCA associated vasculitis. The aim is to establish an animal model for PR3-ANCA associated vasculitis in order to explore the pathogenic role of anti-PR3 antibodies.

Methods: To establish an autoimmune response to rat PR3 Wistar Kyoto (WKY) rats ($n = 4$) were immunized with the following antigens: six chimeric human/mouse PR3 (HHm, HmH, mHH, mmH, mHm, HmH), that are partly composed of the human PR3 (H) amino acid sequence and partly of the mouse PR3 (m), human PR3 (hPR3) and mouse PR3 (mPR3). Ten μ g of protein was administered i.p. in complete Freund's adjuvant with one boost immunization in incomplete Freund's adjuvant. Circulating antibodies to mPR3, hPR3 and rat granulocytes were monitored prior to and 3, 6 and 8 weeks after immunization by direct ELISA (for hPR3 and mPR3) and indirect immunofluorescence on rat white blood cells (for anti-rat PR3). The rats were sacrificed at 8 weeks.

Results: Rats immunized with chimeric human/mouse PR3 proteins recognize rat granulocytes, especially after immunization with the chimeric proteins HHm, HmH, mmH and mHm. Sera of these rats only recognized rat granulocytes and not lymphocytes. Antibodies to hPR3 were induced by immunization with all chimeric proteins and hPR3, but not with mPR3. Antibodies to mPR3 developed especially after immunization with HmH, mmH and HmH, but not after immunization with mPR3.

Conclusion: Immunization with chimeric human/mouse PR3 proteins indeed leads to an autoimmune response to rat granulocytes in rats. Also antibodies to hPR3 and mPR3 developed. The antigen specificity of the anti-rat granulocyte antibodies is currently under investigation.

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Mouse Neutrophil Antigen Distribution May Explain Why Murine Vasculitis Models Are Difficult to Establish

M. Relle, P.R. Galle, A. Schwarting

University Hospital of Mainz, I. Medical Clinic, Mainz, Germany

Objectives: Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies found in the serum of patients with pauci-immune necrotizing and crescentic glomerulonephritis, and systemic small-vessel vasculitis. The neutrophilic granule proteins proteinase 3 (PR3) and myeloperoxidase (MPO) have been identified as the primary target antigens against which ANCA are directed in these disorders. Although there are an increasing number of murine models with similarity to ANCA-positive human diseases, a convincing in vivo model is still lacking.

Methods: For this reason, we generated a peptide antibody against murine proteinase 3 (see figure) and assessed the expression and enzymatic activity of MPO and PR3 in mouse bone marrow and peripheral blood, as well as the expression of this antigen in the kidney and spleen of several autoimmune mouse strains. Mouse MPO was detected in blood and bone marrow by its enzymatic activity. We also analyzed the proportion of neutrophils in the peripheral blood of five frequently used mouse inbred strains.

Results: Our data show that the murine and human myelopoietic systems differ significantly with respect to the two antigens: MPO

Figure PR3 Expression in bone marrow (left) and blood (right)

and PR3 are found only in small amounts in mouse peripheral blood, whereas they are present at high levels in humans. Furthermore, we could demonstrate that mouse bone marrow is a reservoir for functional neutrophils, which are released in the bloodstream a few minutes after injection of IL8. In the case of an infection, this 'neutrophil burst' may protect 'neutropenic' FVB mice from sepsis, due to their low amount of neutrophils in the blood.

Conclusion: Taken together, we have shown that mouse peripheral blood neutrophils lack important functions of their human counterparts and that the maturation processes of mouse neutrophils differ from those of human granulocytes. These facts should be kept in mind when generating infectious disease or autoimmune mouse models.

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Induction: of Pauci-Immune Necrotizing and Crescentic Glomerulonephritis in Myeloperoxidase (MPO) Knockout Mice by Transplantation of MPO+/+ Bone Marrow (BM)

A. Schreiber, H. Xiao, R.J. Falk, J.C. Jennette

University of North Carolina, Nephropathology, Chapel Hill, USA

Background: In vitro studies and animal models suggest that MPO-positive neutrophils and monocytes are the primary pathogenic targets for ANCA. However, there has been no direct in vivo evidence for an interaction between ANCA and neutrophils/monocytes; and the endothelium has been proposed as an alternative primary target. To dissect the contribution of local versus BM-derived cells, we developed a BM-transplantation model for ANCA-disease.

Methods: MPO^{-/-} mice were immunized with murine MPO ($n = 12$). These mice were lethally irradiated (900 rad total body dose) and then reconstituted with 1×10^7 BM-cells from wild type B6 mice (MPO^{+/+}). Control MPO^{-/-} mice ($n = 3$) were immunized with MPO, irradiated and transplanted with MPO^{-/-} BM. Serum anti-MPO-titer, MPO^{+/+} bone-marrow engraftment, and urine protein and blood excretion were monitored. Mice were euthanized at 4 weeks or 8 weeks after BM-transplantation. Kidneys were examined by light and immunofluorescence microscopy.

Results: Of the 12 mice that received MPO^{+/+} BM, 9 had major engraftment with >80% MPO⁺ circulating neutrophils. These 9 mice developed hematuria and proteinuria; and histological

examination revealed glomerular crescents ($14.4\% \pm 6.3$, $7.4-26.4\%$) and necrosis ($5.4\% \pm 1.9$, $2-7.6\%$), with similar severity at 4 and 8 weeks. Immunofluorescence showed a paucity staining for IgG, IgM, IgA, and C3. Three mice that received MPO+/+ BM had minor engraftment with $<50\%$ MPO+ circulating neutrophils and developed no glomerular necrosis or crescents. Control MPO-/- mice that received MPO-/- BM did not develop glomerular necrosis or crescents.

Conclusion: These studies demonstrate that BM-derived MPO+ cells (i.e. neutrophils and monocytes) are the primary target for anti-MPO and are sufficient for inducing glomerulonephritis.

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A Novel Mouse MPO-ANCA Associated Glomerulonephritis

W. Yumura, M. Itabashi, A. Ishida-Okawara, J. Yamashita, Y. Kanashiro, K. Suzuki

Tokyo Women's Medical University, Medicine, Kidney Center, Tokyo, Japan

It has been shown that myeloperoxidase (MPO) and the MPO-specific anti-neutrophil cytoplasmic auto-antibody (MPO-ANCA) are risk factors for the development of these lesions possibly. Using MPO-deficient mice it has been clarified that MPO is a major antigen for MPO-ANCA production. In the sera of patients with microscopic polyangiitis and crescentic glomerulonephritis (CrGN), high titers of MPO-ANCA are frequently detected. As the basis for clinical studies, animal models are often used to understand the mechanisms of the development of vasculitis, and to establish therapeutic strategies. Both MRL lpr/lpr, and SCG/Kj strains are known to show high levels of MPO-ANCA in association with renal lesions including glomerulonephritis (GN) and vasculitis. The role of activated neutrophils in the development of nephritis in SCG/Kj mice have been evaluated on the relationship between neutrophil functions and renal lesions developing spontaneously. However, to know pathogenic roles of MPO-ANCA and neutrophils and the development of therapeutic conditions for GN and vasculitis, events occurred in renal failure must be investigated using induction model mice. We studied here on preparing animal model for ANCA-related GN induced with bovine serum albumin (BSA).

Materials: BSA (0.2 mg) was injected intraperitoneally into C57BL/6N 7-week old female mouse four times for 8 weeks. Subsequently, BSA (50 mg/kg) was injected everyday for 6 weeks.

Results & Discussion: Body weight loss, appearance of hematuria, increase of BUN, platelets, neutrophils and monocytes in peripheral blood were observed. Titer of MPO-ANCA in serum markedly increased during clinical course. We also observed histologically the development of in some mice from proliferative GN to CrGN with neutrophils infiltration into the renal glomeruli. Finally, 75% of the mice injected with BSA showed CrGN at 14-week stage. These findings indicate that MPO-ANCA and activated neutrophils may be associated with development of CrGN by administration with serial BSA. This model mice will be useful for good model CrGN for the establishing therapeutic strategies.

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c-ANCA Induce Neutrophil-mediated Lung Injury in the Isolated Rat Lung – An Experimental Model of Acute Wegener's Granulomatosis

K. Hattar, S. Oppermann, U. Grandel, B. Schmitt, N. Weissmann, R. Schermuly, W. Seeger, F. Grimminger, U. Sibellus

University of Giessen, Dept. of Internal Medicine, Giessen, Germany

Anti-neutrophil-cytoplasmic antibodies (c-ANCA) targeting Proteinase-3 (PR3) are implicated in the pathogenesis of Wegener's Granulomatosis (WG). Fulminant disease can present as rapid-progressive glomerulonephritis and/or acute lung injury. In the current study, we developed a model of acute lung injury in WG using isolated perfused and ventilated rat lungs. A pathogenic role of c-ANCA in lung injury could be established. Isolated human polymorphonuclear leukocytes (PMN) were primed with TNF-alpha to induce surface expression of PR3. Co-perfusion of TNF-primed neutrophils and monoclonal anti-PR3-antibodies (anti-PR3) induced a massive weight gain in isolated rat lungs. This effect was not observed when isotype-matched control-IgG (IgGc) was co-perfused with TNF-primed PMN. ANCA-induced edema formation was paralleled by a raise in the capillary filtration coefficient (kfc) as a marker of increased pulmonary endothelial permeability. In contrast, pulmonary artery pressure (PAP) was not affected by perfusion with anti-PR3-antibodies. In the presence of the oxygen radical scavenger superoxide-dismutase (SOD) and a specific neutrophil NADPH-oxidase inhibitor, ANCA-induced lung edema could be prevented. Inhibition of neutrophil elastase had a partial effect in preventing ANCA-induced lung injury. In conclusion, anti-PR3-antibodies induce neutrophil mediated, oxygen radical dependent acute lung injury in the model of the isolated rat lung. This experimental model supports the hypothesis of a pathogenic role of c-ANCA in WG and offers the possibility to develop therapeutic strategies for the treatment of lung injury in fulminant WG.

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Strain Dependency of CAWS-induced Coronary Arteritis in Mice

N. Ohno, N.N. Miura, H. Shihonara, H. Sankawa

Tokyo Univ. Pharm. Life Sci., School of Pharmacy, Tokyo, Japan

CAWS is a water-soluble extracellular polysaccharide fraction obtained from *Candida albicans*. CAWS shows characteristic pathological effects in vivo in mice, such as acute lethal shock and arteritis induction. CAWS also shows various biological activities in vitro, such as platelet aggregation, complement activation, and modulation of proclotting activity of endothelial cells. However, molecular mechanisms of these biological effects are largely unknown. In the present study, strain dependency of CAWS-induced coronary arteritis resembled to Kawasaki disease and underlying mechanism were investigated.

As regard to repeated intraperitoneal administrations of CAWS, various strains of mice, such as AKR/N, A/J, C3H/HeJ, CBA/N, DBA/1, DBA/2, BALB/c were found to be sensitive to induce coronary arteritis. In contrast, CBA/J mice showed resistant. The coronary arteritis of DBA/2 mice was the most serious, with majority of mice expired about 6 weeks from CAWS administration. Within the observation period, hyperplasia started around 1 week and heart weight increased around 3 weeks from CAWS administration. CAWS-induced coronary arteritis was induced by only 1/10 dose of standard protocol and also by intravenous administration. Stimulation of spleen cells of naive DBA/2 mice by CAWS in vitro enhanced release of GM-CSF, but not in other strains. In addition, spleen cells of CAWS-administered DBA/2 mice released significant amount of IFN- γ , IL-6, and TNF- α in response to CAWS. Addition of enough amount of GM-CSF, spleen cells of naive DBA/2 mice released significantly higher amount of IFN- γ , IL-6 and TNF- α in response to CAWS. These facts strongly suggest that CAWS-induced GM-CSF productivity would be a key property for severe coronary arteritis in this strain. The CAWS-induced coronary arteritis model is a good tool for analyzing molecular mechanism and development of new therapeutic strategy for arteritis.

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Beta-glucan of Candida Cell Wall Aggravate Murine Model of Autoimmune Disease

S. Hida, N.N. Miura, Y. Adachi, N. Ohno

Tokyo Univ. Pharm. Life Sci., School of Pharmacy,
 Tokyo, Japan

Diseases related to autoimmunity are regulated by various factors, such as genetic background, gender, environment, microbial flora, and infectious disease. The principal factors of the disease would be varied in each other. We are interested in the relationship between fungal metabolite and autoimmune diseases, and already found that Candida water soluble polysaccharide fraction, CAWS, significantly induced coronary arteritis resemble to Kawasaki Disease¹). Fungal metabolites would be related to other autoimmune diseases. Collagen-induced arthritis (CIA) is an animal model of rheumatoid arthritis (RA) and one of the best characterized one. In the present study, we examined the effect of fungal metabolites to CIA model. In the standard induction Methods, Type II collagen (CII) emulsified with Freund's complete adjuvant (FCA) is injected into DBA/1 mouse. After booster injection, the mouse developed inflammation of their paws. In the present study, CAWS and beta-glucans derived from *Candida albicans* were tested as adjuvant to induce CIA. DBA/1 mice

were injected CII with FCA on day 0 and boosted at day 21. These mice developed arthritis around 7–10 days after booster. When CAWS was used as adjuvant, no signs of inflammation could be detected. However, mice immunized CII with OX-CA, particulate form of beta-glucan, and CSBG, solubilized beta-glucan showed severe arthritis score. Histological changes and anti-CII antibody production were similar to the symptoms shown by CII with FCA. It is of note that the arthritis induced by OX-CA showed the worst score. These facts strongly suggested that fungal metabolites such as CAWS and beta-glucans have capacity to induce and modulate autoimmune diseases, such as vasculitis and arthritis.

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Human Anti-neutrophil Cytoplasm Autoantibodies to Proteinase 3 (PR3-ANCA) do Bind to Neutrophils

A. van Rossum, Y.M. van der Geld, P.C. Limburg,
 C.G.M. Kallenberg

University Medical Center Groningen, Rheumatology and
 Clinical Immunology, The Netherlands

Objective: The in vivo pathogenic role of anti-neutrophil cytoplasm autoantibodies (ANCA) in ANCA-associated vasculitis is being challenged by a recent report suggesting that ANCA directed against proteinase 3 (PR3) cannot bind to its target autoantigen PR3 on circulating neutrophils (PMN). In the present study we explored binding of human PR3-ANCA to membrane bound PR3 on PMN.

Methods: Serum or plasma from PR3-ANCA positive patients with Wegener's Granulomatosis (WG) (n = 8) or healthy controls (n = 8) were incubated with TNF α -primed PMN or PMA-stimulated PMN from donors showing bimodal PR3 expression on their PMN membrane (n = 3). Binding of IgG from these sera or plasma samples was assessed by indirect immunofluorescence.

Results: Binding of IgG in undiluted plasma or serum from PR3-ANCA positive WG-patients to PMN was significantly increased compared to plasma or serum from healthy controls. Dilution of plasma and serum showed typical concentration-dependency of IgG binding. Binding was specific since IgG from plasma or serum from PR3-ANCA positive patients only bound to PMN that expressed PR3 and not to PMN that lacked PR3 expression as assessed by double staining.

Conclusion: PR3-ANCA in undiluted serum or plasma from PR3-ANCA positive WG patients do bind to TNF α -primed or PMA-stimulated PMN that express PR3 on their membrane.

Anti-neutrophil Cytoplasm Antibody Associated Vasculitis Disease Remission Is Associated with Changes in Neutrophil and Peripheral Blood Lymphocyte Subsets and Function

M.D. Morgan, M.D.T. Drayson, L. Harper

University of Birmingham Medical School,
Renal Immunobiology, Birmingham, United Kingdom

Patients with ANCA associated vasculitis (AAV) have abnormal proportions of peripheral blood lymphocyte subsets and cytokine primed neutrophils, compared to normal controls. The effect of immunosuppression on in vivo neutrophil priming and lymphocyte subsets in patients with AAV was investigated in the setting of a safety and efficacy pilot study of Infliximab. 25 patients with AAV received standard immunosuppression (SI) ($n = 13$), or SI and 4 doses of Infliximab ($n = 12$) over 10 weeks. C-reactive protein (CRP) and Birmingham vasculitis activity score (BVAS) were recorded over 14 weeks. Lymphocyte subsets and neutrophil respiratory burst response to fMLP (ratio burst response fMLP: control) were monitored by whole blood flow cytometry. All 25 patients had active disease with a median BVAS of 11 (4–12) and CRP 27 (0–168) at entry. After 14 weeks, 80% of patients had BVAS 0/1 and all had CRP < 10. Added Infliximab does not reduce the time to remission. The neutrophil respiratory burst response to fMLP is reduced after 6 weeks of infliximab therapy (1.26 vs 1.09 $p = 0.02$) but unchanged in the SI group (1.26 vs 1.23). Comparing all AAV ($n = 25$) with healthy controls ($n = 17$), expression of the B memory cell marker CD27 was decreased in active vasculitis and increased in remission with no difference in B cell expression of CD23. The expression of CD25 on CD4+ and HLA-DR on CD8+ T-cells was increased in AAV. CD25 expression on CD4+ cells increased with remission. The proportion of CD28neg CD4+ T-cells (TNF and IFN – producing cells) was increased in vasculitis with a trend to reduction with remission (table 1). Neutrophil respiratory burst response to fMLP is reduced after Infliximab, which may represent reduced in vivo neutrophil priming. The proportion of CD27 expressing B-cells is reduced in acute disease and increases with remission. CD25 expression on CD4+ T-cells increases with disease remission and may represent an increase in T-regulatory cells but needs further investigation.

Table 1. The percentage expression of CD25, HLA-DR & CD28 on CD4+ and CD8+ T-cells and CD23 and CD27 expression on CD19+ B-cells

Lymphocyte markers		Normal donors ($n = 17$)	Vasculitis patients ($n = 25$)	
			Week 0	Week 14
CD4	CD25	52	58*	73*
	HLA-DR	6	7	9
	CD28neg	2**	7**	4
CD8	CD25	7	6	11
	HLA-DR	9***	19***	29
CD19	CD23	57	51	50
	CD27	30	19****	45****

* $p = 0.05$ ** $p = 0.02$ *** $p = 0.05$ **** $p = 0.007$

The Inducible Co-stimulatory Molecule (ICOS) and ICOS-ligand Pathway in Wegener's Granulomatosis: Expression and Functional Analysis

K. de Groot, B. Hellmich, A. Müller, R. Kroczeck, H. Haller, W.L. Gross, E. Csernok

Medical School Hannover, Nephrology, Hannover, Germany

Objective Patients: Since patients with Wegener's granulomatosis (WG) lack expression of the co-stimulatory molecule CD28 on T cells, the aim of this study was to characterize the expression of an alternative T cell costimulatory pathway that involves the inducible co-stimulatory molecule (ICOS) and its ligand (ICOS-L).

Methods: We used flow cytometry to analyze expression of the T cell-specific ICOS and its ligand (ICOS-L) on monocytes and B cells obtained from the peripheral blood of WG patients ($n = 10$) and healthy controls (HC, $n = 10$). Th1/Th2 cytokine profiles were analyzed after in vitro activation of CD3/CD28 and ICOS pathway by flow cytometry using cytometric beads array. In addition, WG nasal tissues were analyzed by immunohistology.

Results: There was no significant difference in the ICOS expression pattern on CD4+ as well and CD8+ T cells between WG and HC in peripheral blood. Based on in vitro experiments, we documented a down-regulation of ICOS expression on CD8+ cells in WG in comparison to HC (0.26% vs. 0.49%, $p = 0.038$) and a lower percentage of monocytes was ICOS-L+ in WG (0.79% vs. 1.14%, $p = 0.015$). A significantly higher expression of ICOS-L was detected on B cells from WG compared to HC after in vitro activation (21.53% vs. 9.65%, $p = 0.021$). After in vitro activation of CD3 and ICOS pathway no difference in cytokine production (IFN- α , IL-2, IL-4, IL-5 and TNF- α) was detected between WG and HC, except reduced IL-10 levels in patients with generalized WG (903.03 pg/ml vs. 523.20 pg/ml, $p = 0.02$). The ICOS molecule was detected in the nasal biopsies of WG patients.

Conclusion: The study identifies ICOS and its ligand as an alternative co-stimulatory pathway involved in T cell activation in WG. The expression pattern of co-stimulatory molecules ICOS and the co-stimulatory activity of CD3 and ICOS in WG were similar to that in HC. WG patients show an altered expression profile of ICOS-L: a decreased expression of ICOS-L on monocytes and a preferential up-regulation on B cells ex vivo as well as after in vitro activation. The importance of these unusual expression patterns for the pathogenesis of WG remains to be clarified.

Interleukin 18: A Pro-inflammatory Cytokine in Renal ANCA Associated Vasculitis

M.D. Morgan, P. Hewins, C.O.S. Savage, D.A.H. Neil, L. Harper

University of Birmingham Medical School,
Renal Immunobiology, Birmingham, United Kingdom

Interleukin 18 (IL-18) is a recently described pro-inflammatory cytokine. Previously we have described its presence in renal tissue and its ability to prime the ANCA induced neutrophil respiratory burst response in a time and dose dependent manner.

Objectives: To identify the cells producing IL-18 in the kidney and to investigate the signalling pathways involved in neutrophil priming.

Methods: IL-18 producing cells in the kidney were identified by dual staining fluorescent immunohistochemistry and confocal microscopy. Staining, in vasculitis and normal renal tissue, was scored on a 4 point arbitrary scale by an independent pathologist. Signalling pathways were investigated by flow cytometry of primed neutrophils and ANCA induced superoxide production in the presence of anti-TNF antibodies or a p38 MAPK inhibitor.

Results: Compared to normal control renal tissue, increased IL-18 was seen in patients with ANCA associated vasculitis. IL-18 staining for vasculitis vs control biopsies was: glomerulus 2.2 ± 0.4 arbitrary units vs 0.6 ± 0.5 ($p < 0.001$), interstitium 2.7 ± 0.8 vs 0.5 ± 0.4 ($p < 0.001$) and distal tubules 1.4 ± 0.5 vs 0.4 ± 0.5 ($p = 0.01$). Within the glomerulus IL-18 colocalised with synaptopodin (podocytes) but not smooth muscle actin (SMA) or von Willebrand factor (VWF) (activated mesangial and endothelial cells). Outside the glomerulus IL-18 was seen in distal convoluted tubules and also colocalised with CD68 (macrophages) and SMA (myofibroblasts) but not VWF. IL-18 primed neutrophils had increased expression of activation markers CD11b/CD18 and shed L-selectin but did not alter their surface expression of ANCA antigens proteinase 3 or myeloperoxidase. Inhibition of p38 MAPK with SB203580 abrogated the IL-18 priming of the respiratory burst in a dose dependent manner. TNF blockade did not affect respiratory burst confirming that IL-18 priming is independent of TNF.

Conclusion: IL-18 is potentially an important pro-inflammatory cytokine in AAV. It is upregulated in resident renal cells driving inflammation from within the kidney and infiltrating macrophages. It primes the ANCA induced neutrophil respiratory burst via phosphorylation of p38 MAPK without increasing the surface expression of the ANCA antigens MPO and PR3.

Clinical Relevance of Persistently Elevated Circulating Cytokines (TNF Alpha and IL-6) in the Long-term Follow-up of Patients with Giant-cell Arteritis (GCA)

A. García-Martínez, J. Hernández-Rodríguez, X. Filella, J.M. Grau, M.C. Cid

Hospital Clínic, Internal Medicine, Barcelona, Spain

Background: Corticosteroid therapy is highly effective in patients with GCA. However, it has been shown that cytokines such as IL-6 and endothelial molecules such as vWFAg may remain moderately elevated during follow-up, suggesting persistence of residual vascular inflammatory lesions.

Objectives: To assess the clinical impact of elevated cytokines (TNFa and IL-6) in the long-term follow-up of patients with GCA.

Patients and Methods: Prospective evaluation of 26 patients (8 M and 18 F) with biopsy-proven GCA, treated and followed by the authors for a median of 6.2 years (range 4.4–9.8). Patients were subjected to clinical examination and additional data recorded included present daily prednisone dose, time required to achieve a maintenance prednisone dose < 10 mg/day and number of relapses. Patients performed a self-evaluation of quality of life (pain, physical activity and general well-being) by means of a visual scale (from 0 to 10). Circulating IL-6 and TNFa were measured by ELISA. Acute-phase reactants (ESR, CRP and haptoglobin) were also determined.

Results: All patients were in clinical remission. Six patients (23%) had not presented disease flares during the follow-up period, whereas 7 (27%) had experienced 1 relapse and 13 (50%) had presented more than one. No patients developed disease-related complications during follow-up. At the time of the evaluation 16 patients (61%) had successfully discontinued prednisone whereas 9 patients (39%) were still under low-dose prednisone treatment (median dose 3.75 mg/day, range 1.25–7.5). Median IL-6 was 11 pg/ml (range 0–84) and median TNFa was 32 pg/ml (range 13–59). 65% of patients had IL-6 levels above the normal range (values 0–5 pg/ml) and 81% of patients had increased TNFa levels (normal values 0–20 pg/ml). TNFa levels correlated with IL-6 concentrations ($p = 0.032$) but not with ESR, CRP and haptoglobin. No differences in cytokine levels were found in patients receiving no treatment compared with those still requiring corticosteroids and no relationship was found between cytokine levels and patients' perceived quality of life at the time of evaluation. However, TNFa levels correlated with the time required to reach a maintenance prednisone dose below 10 mg/day ($p = 0.044$) and with the relapse rate ($p = 0.025$).

Conclusions: In this small cohort of patients, elevated circulating TNFa or IL-6 levels after long-term follow-up do not appear to have a major clinical impact. However, TNFa persists higher in patients who had experienced a more relapsing disease with higher prednisone requirements. As life-expectancy increases, larger studies are needed to assess the long-term clinical consequences of persistent inflammatory activity.

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Vasculitis and Peroxiredoxin Autoimmunity

S. Ozaki, R. Karasawa, S. Ooka, M. Fujieda,
K. Nishioka, T. Kato

St. Marianna University, Internal Medicine,
Kawasaki, Japan

Objectives: We tried to identify target antigens of anti-endothelial cell antibodies (AECA) by a proteomic technique. We also investigated the clinical importance of the identified autoantigens.

Methods: To detect autoantigens for AECA, we extracted proteins from human umbilical cord vein endothelial cell (HUVEC) and HeLa cells, separated them by a 2-dimensional electrophoresis, and transferred them onto membranes, which were subjected to western blotting (WB) using serum samples of patients with systemic vasculitides. We detected protein spots that reacted selectively in the HUVEC samples, identified the autoantigens by a mass finger-printing technique, prepared their recombinant proteins, and confirmed their antigenicity.

Results: We detected 51 HUVEC-specific autoantigens, one of which was found peroxiredoxin II (Prx II), an anti-oxidative enzyme. We found that 60% of the serum samples of patients with systemic vasculitides were positive, whereas only 4% were positive in the patients without vasculitis. In particular, 86% and 50% of serum samples of patients with Takayasu arteritis and Kawasaki disease were positive, respectively. The antigenicity of Prx II was found different from that of its related enzymes Prx I and IV. Indirect immunofluorescence revealed the existence of Prx II on the cell surface of HUVEC. Clinically, titers of D-dimer and TAT were found significantly higher in the anti-Prx II-positive patients than the negative ones, and anti-Prx II titers changed in parallel with disease activity.

Conclusions: The autoantibody to Prx II, which we found for the first time, would be a useful diagnostic marker for systemic vasculitides, in particular for Takayasu arteritis and Kawasaki disease. Clinical significance of this antibody seemed to be related to the thrombin formation. This autoantibody may play a pathological role by inhibiting anti-oxidative activity of Prx II by binding them on the endothelial cell surface.

Genes Related to the Maintenance of Inflammatory Lesions in Giant-cell Arteritis. Association between Increased CCL2 (MCP-1) Expression and Persistence of Disease Activity

M.C. Cid, M.P. Hoffman, J. Hernández-Rodríguez,
M. Segarra, M. Elkin, M. Sánchez, C. Vilardell,
A. García-Martínez, M. Pla-Campo, A. Urbano-Márquez,
J.M. Grau, H.K. Kleinman

Hospital Clínic, Internal Medicine, Barcelona, Spain

Background: Patients with giant-cell arteritis (GCA) who develop a strong systemic inflammatory response are more refractory to corticosteroid treatment.

Methods and Results: Gene expression profiles were analyzed with cDNA arrays in temporal artery samples from 6 patients with strong and 6 patients with weak systemic inflammatory response in order to identify genes potentially related to the persistence of inflammatory lesions. CCL2 (MCP-1) was one of the genes up-regulated in patients with strong systemic inflammatory response and it was subsequently measured by real-time quantitative PCR in temporal arteries from 35 patients with GCA and 9 controls. CCL2 mRNA was significantly higher in patients than in controls (31 ± 15.6 vs 0.44 ± 0.10 , $p = 0.0001$). In addition, CCL2 was more abundant in patients who experienced 2 or more relapses during the first year compared to those who endured sustained remission (127 ± 82 vs 11 ± 5.5 , $p = 0.0233$) and correlated with the cumulated prednisone dose ($r = 0.533$, $p = 0.0024$). The time required to achieve a maintenance prednisone dose lower than 10 mg/day was longer in patients with CCL2 levels higher than 3 units ($p = 0.0173$). Additional genes differentially expressed included genes related to monocyte recruitment (MPR-8), cell migration (RhoA, RhoB, PRL-1), cell stress (RAD52, p27 HSP), and, interestingly, genes related to neural development (neuronatin), neurotransmission (neuromedin K receptor) and bone remodelling (OSF) with unanticipated functions in inflammation or vascular biology.

Conclusion: CCL2 (MCP-1) expression is associated with persistence of disease activity in GCA.

Circulating Inflammatory Endothelial Cells Contribute to Endothelial Progenitor Cell Dysfunction in Vasculitis Patients

C. Holmén, P. Stenvinkel, A.R. Qureshi, E. Elsheikh,
E. Pettersson, S. Jalkanen, S. Sumitran-Holgersson

Karolinska Institute, Clinical Immunology,
Stockholm, Sweden

Objectives: Using Wegener's granulomatosis (WG) as a study model, we examined if circulating inflammatory endothelial cells (IECs) could; i) be phenotypically distinguished from endothelial progenitor cells (EPCs) ii) be used as a disease/injury activity marker iii) contribute to sustained vascular damage by inducing endothelial progenitor cell dysfunction.

Methods: Two endothelial-associated inflammatory molecules, vascular-adhesion protein-1 (VAP-1) and MHC class I-related chain A (MICA) were tested to differentiate IECs from EPCs by flow cytometry and immunocytochemistry. Various immunoassays were used to study the functional role of IECs isolated from WG patients with active disease ($n = 16$), in remission ($n = 20$) including healthy controls ($n = 20$). Various chemokines produced by cultured IECs were detected with standard ELISA and expression of VAP-1 and MICA in WG kidney biopsies was examined by immunohistochemistry.

Results: IECs but not EPCs expressed VAP-1 and MICA. IECs were significantly increased in patients with active disease as compared to those in remission ($p < 0.001$). IECs expressed high levels of iNOS, neutrophil activating chemokines such as MIP-1a, GRO-a, ENA-78 and IL-8 ($p < 0.008$) and induced increased neutrophil migration ($p < 0.008$). IEC levels significantly correlated with C-reactive protein ($p = 0.01$) and extent of organ involvement ($p = 0.001$). Patients with

active disease showed decreased numbers of EPC colony-forming units ($p = 0,006$) and a high expression of VAP-1 and MICA in kidney endothelium. IECs significantly inhibited proliferation ($p < 0,008$), migration ($p < 0,008$) and eNOS expression in EPCs.

Conclusion: Apart from being a new disease activity marker, IECs may contribute to vascular damage by impairing the functional capacity for repair by EPCs as well as contribute to sustained vascular damage by inducing endothelial progenitor cell dysfunction.

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A Promotor Haplotype Associates with a Pulmonary Phenotype in ANCA Vasculitis

C. Nester, J.C. Edberg, R.P. Kimberly, K. Wilhelmsen, J.C. Jennette, R.J. Falk

University of North Carolina, Nephrology,
Chapel Hill, USA

We hypothesize that pulmonary involvement in ANCA vasculitis is attributable to polymorphisms in the FCGR2B gene. The rationale for this hypothesis is based on data from our animal studies indicating that *Fcgr2b*^{-/-} mice immunized with anti-myeloperoxidase (MPO) antibodies develop a pulmonary, granulomatous and hemorrhagic phenotype (abstract SA-FC075: Xiao; ASN 2004). Further support is provided by reports that a polymorphic haplotype in the promoter region of this gene is a risk factor for another autoimmune disease, human lupus erythematosus in Caucasians (Su, JI: 7186). One hundred and fifty Caucasian patients diagnosed with ANCA vasculitis (80 PR3/70 MPO) and categorized as renal limited (3 PR3/8 MPO), with pulmonary involvement (54 PR3/32 MPO), or without pulmonary involvement (23 PR3/30 MPO) were genotyped by pyrosequencing for the -120A/T and -386G/C alleles. The data indicate that 20.9% of patients with pulmonary involvement are of the haplotype 120A/-386C versus only 6.6% of the nonpulmonary ANCA patients ($p = 0.01$). The prevalence of this haplotype in the general population (9.4%) was established using healthy controls from the Carolina Lupus Study. These data indicate an overrepresentation of the less frequent haplotype in the pulmonary ANCA patient population vs healthy controls ($p = 0.004$). An additional 150 ANCA patients are being genotyped to increase the power of these results. The polymorphic haplotype in the promoter region of this gene that codes for this inhibitory Fc receptor, Fc-IIb, may play a role in increased risk of a pulmonary phenotype in ANCA vasculitis.

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Antibodies to Human Lysosomal Membrane Glycoprotein 2 (HLAMP2) and Bacterial Peptides in ANCA Associated Focal Necrotising Glomerulonephritis (FNGN)

C.A. Low, M. Exner, R. Brandes, S.R. Henderson, P. Klemm, D. Kerjaschki, R. Kain

University of Aberdeen, Pathology, Aberdeen,
United Kingdom

We originally identified human lysosomal membrane protein2 (hlamp2) as novel ANCA target in a systematic search for ANCA antigens expressed on the surface of endothelial cells and neutrophilic granulocytes. Here we investigated the prevalence and specificity of anti-hlamp2 antibodies and whether they might be pathogenic. We produced hlamp2 fusion proteins to develop robust ELISAs and analysed anti-hlamp2 antibodies in two large cohorts of 48 patients each with ANCA associated FNGN from Vienna and Aberdeen. Control groups were 88 patients with other forms of vasculitis or glomerular disease and 52 healthy volunteers. Overall anti-hlamp2 antibodies are present in 97% of untreated patients in addition to either MPO or PR3, and the antibody titres correlated with quantitative scores of disease activity in renal biopsies. The prevalence of hlamp2 antibodies was much lower in patients with inactive diseases (22%) and they were found at low titre only in 4% of healthy controls. 19 of the 88 disease controls that had anti-hlamp2 antibodies also had focal necrosis or crescents and ANCA by indirect immunofluorescence. Consequently we established that IgGs in most ANCA sera of patients with active FNGN bound to three peptides on the protein backbone of hlamp2. One peptide has homology to fimH, a fimbrial mannose binding protein of gram negative pathogens. Crossreactivity of anti-hlamp2 and anti-fimH antibodies was confirmed by pre-incubating hlamp2 positive sera with crude lysates from relevant and unrelated bacteria, recombinant fimH, cross-reactive and unrelated peptides in a series of systematic competition ELISAs. Binding to MPO or PR3 was not competed by either fimH or hlamp2. Finally, we investigated the potential pathogenicity of anti-lamp2 antibodies by immunising WKY rats with fimH alone or in combination with lamp2. Rats immunised with either antigen developed ANCA associated FNGN, pulmonary vasculitis and fimH antibodies that cross-react with lamp-2. Our results show that autoantibodies to hlamp2 are closely associated with FNGN both, clinically and in a rat model. Cross-reactivity between hlamp-2 and fimH raises the possibility that molecular mimicry plays an aetiological factor in FNGN and that the immune response to fimH after infection breaks tolerance to hlamp2 in susceptible individuals.

Association of Thyroid Disease and its Treatment with the Onset of ANCA Small Vessel Vasculitis

S.L. Hogan, M.S. Joy, H. Chin, J.C. Jennette, R.J. Falk, P.H. Nachman

University of North Carolina, Nephrology & Hypertension, Chapel Hill, USA

Case reports and small case series have described onset of ANCA-small vessel vasculitis (SVV) with use of anti-thyroid agents, most commonly propylthiouracil (PTU). This study assessed the association of thyroid disease with ANCA-SVV in a southeastern US population-based case-control study. Cases ($n = 129$) had ANCA-SVV with biopsy-proven glomerular involvement. Community-based controls ($n = 99$) were frequency matched by gender and age. Use of specific drugs and co-morbidities prior to ANCA-SVV diagnosis were assessed by telephone interview. Logistic regression controlling for age, gender and state was used to evaluate associations with ANCA-SVV. Adjusted odds ratios (OR) and 95% confidence intervals (CI) are reported. Since thyroid disease is more common in women, estimates limited to females, controlling for age and state, are also reported. History of any thyroid disease was reported by 27 (21%) of cases and 7 (7%) of controls (OR = 4.0, CI 1.6–10.2, $p = 0.003$), and among females by 21/52 (40%) of cases and 5/53 (9%) of controls (OR = 5.6, CI 1.8–16.9, $p = 0.002$) (In the US, about 10% of adults have abnormal thyroid hormone levels). Use of anti-thyroid agents was reported in 2 female cases (1 PTU, 1 methimazole) and 0 controls (OR not calculable). Use of thyroid replacement was reported by 20 (16%) cases and 6 (6%) controls (OR = 3.3, CI 1.2–8.9, $p = 0.02$), and among females by 17 (33%) cases and 4 (7.5%) controls (OR = 5.1, CI 1.5–17.2, $p = 0.008$). One case reported thyroid cancer and 3 cases and 1 control did not specify a thyroid disease or treatment. There was more MPO-ANCA (75%) than PR3-ANCA (25%) in cases with thyroid disease compared to cases without thyroid disease (46% and 54%, $p = 0.02$). Anti-thyroid agents account for little of the overall incidence of ANCA-SVV. Thyroid disease in general, and specifically hypothyroidism and/or thyroid replacement therapy, is associated with ANCA-SVV, most commonly MPO-ANCA, and may reflect common underlying autoimmune propensity.

Sphingomyelinase as a Link between Vascular Inflammation and Atherosclerosis

L.D. Church, A. Coney, C. Ray, S.P. Young, J. Marshall, P. Bacon

University of Birmingham, Rheumatology, Birmingham, United Kingdom

Systemic vasculitis (SV) provides an ideal opportunity to probe the ill-understood mechanisms whereby inflammation promotes atherosclerosis. SV is associated with diffuse endothelial cell dysfunction (ECD), the first step in the complex process of atherosclerosis. Blockade of the inflammatory cytokine TNF α can reverse this ECD.

We recently showed that in human T-cells exposed to TNF, aberrant Ca^{2+} signals after TCR activation are accompanied by sphingolipid release which is blocked in cells from sphingomyelinase knock-out mice. Here we test the hypothesis that sphingolipid mediators may link inflammatory cytokines to the depression of endothelial NO synthase underlying ECD. In rat aorta the effect of SMase and C2-ceramide on NO production to both adenosine and to bradykinin (BK) stimulation was examined ex-vivo using an NO electrode. Endothelium-specific NO production was significantly inhibited. Similar inhibition following pre-treatment with SMase or C2-ceramide was seen using cultured HUVECs in-vitro. eNOS activation is Ca^{2+} dependent, so intracellular calcium signalling ($[\text{Ca}^{2+}]_i$) to BK or thrombin was examined in HUVEC using a single cell imaging system. This revealed depressed $[\text{Ca}^{2+}]_i$ responses to both mediators. To determine the site of the signalling perturbation, we analysed intracellular and transmembrane fluxes in response to the receptor-independent action of thapsigargin. This showed specific inhibition of Ca^{2+} influx across the plasma membrane. Finally the in-vivo relevance of these findings was assessed in a well-documented model of blood-flow. SMase was infused into the distal aorta of wistar rats and endothelium-dependent NO-mediated flow responses to acetylcholine were measured in both the local femoral and the distal brachial artery. Vasodilatation was impaired at both sites within 10 minutes of SMase exposure and persisted for more than one hour. These data are consistent with the novel hypothesis that SMase activated by TNF α can affect receptor-mediated Ca^{2+} signalling and depress NO responses. We propose a model in which high local cytokine concentrations in blood vessel inflammation produces local EC activation – but also releases sphingolipid mediators that can induce downstream ECD and initiate atherogenesis.

Neutrophil Activation in the Initial Step of Arteritis Development Induced with CAWS Injection

A. Ishida-Okawara, N. Nagi-Miura, T. Oharaseki, N. Ohno, K. Takahashi, H. Okamura, P.A. Ward, K. Suzuki

National Institute of Infectious Diseases, Bioactive molecules, Tokyo, Japan

CAWS, *C. albicans* water-soluble mannoprotein-b-glucan complex, which is released into cultured medium, has various biological activities. Moreover, we have established a mouse model system which shows the symptoms of arteritis with MPO-ANCA production by consecutive injections to a mouse. We present about neutrophil activation and inflammatory responses in the initial step after CAWS injection.

Materials and Methods: C57BL/6N mice (male, 6w) were used under SPF condition. CAWS solution sterilely prepared in PBS was intraperitoneally injected into a mouse. During 16 hours, the mice were sacrificed in various time, then profiles of peripheral blood cells were analyzed. In addition, peripheral neutrophil functions, the levels of inflammatory cytokines and ICAM-1 in plasma were measured. On the other hand, peritoneal exudate neutrophil was prepared by injection of 8% casein intraperitoneally to analyze direct effect of CAWS on neutrophil activation in vitro.

Result and Discussion: The number of peripheral leukocytes was immediately increased after CAWS injection, especially, neutrophil

was markedly increased. In addition, degranulation and superoxide generation of neutrophil with FMLP or PMA were enhanced. On the other hand, the levels of inflammatory cytokines, IL-1b, IL-6 and IL-10, and subsequently ICAM-1 in plasma were significantly increased. It was also shown that CAWS directly activated neutrophil to produce IL-1b and IL-10 in vitro. From these findings, neutrophil number was primarily increased in peripheral blood at the initial step after CAWS injection, and CAWS directly activated neutrophil to produce inflammatory cytokines ICAM-1, a marker of tissue injury, was also systemically increased suggests subsequent arteritis development.

Conclusion: We showed a possibility that the production of inflammatory cytokines and subsequent ICAM-1, increase and activation of neutrophil could involve to the early event during the arteritis development.

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The Long Pentraxin PTX3 is Abundantly Present at Sites of Leukocytoclastic Lesions in Patients with Small-Vessel Vasculitis

A. van Rossum, H. Pas, F. Fazzini, M.G. Huitema, P.C. Limburg, M. Jonkman, C.G.M. Kallenberg

University Medical Center Groningen, Rheumatology and Clinical Immunology, The Netherlands

Objective: We previously reported that the prototypic tissue pentraxin PTX3 inhibits phagocytosis of late apoptotic polymorphonuclear leukocytes (PMN) by macrophages. In addition, levels of PTX3 parallel disease activity in small-vessel vasculitis, which is characterized by leukocytoclasia, a phenomenon of accumulation of nuclear remnants from unscavenged PMN in or near the vessel wall. We hypothesize that PTX3 accumulates at sites of leukocytoclastic vasculitis and, as such, can be a key factor for the induction of leukocytoclasia.

Methods: We selected skin biopsy specimens of 13 patients with small vessel vasculitis and of 4 healthy controls. Histopathologically, these specimens were characterized as leukocytoclastic vasculitis. Specimens were studied for the presence of PTX3 using rabbit anti-PTX3 polyclonal antibodies and rat anti-PTX3 monoclonal antibodies (MNB4). Specimens were scored morphometrically for leukocytic infiltration in conjunction with PTX3 staining. Morphometric scores are presented as percentages of total tissue specimen area.

Results: Staining biopsy specimens of patients with leukocytoclastic vasculitis for PTX3 revealed an abundant presence of this pentraxin at sites of leukocytoclastic infiltrates. In biopsy specimens of vasculitic patients significantly more PTX3 ($48.9 \pm 6.1\%$, $n = 13$) was found in the tissue compared to controls ($4.8 \pm 1.5\%$, $n = 4$) ($p = 0.0035$). Furthermore, PTX3 was localized around vessels but could be localized in a diffuse manner throughout the tissue as well.

Conclusion: PTX3 is abundantly present at sites of leukocytoclastic lesions of patients with small-vessel vasculitis, whereas not in controls. Taken together, the inhibitory effect of PTX3 on phagocytosis of late apoptotic PMN by macrophages in combination with its abundant presence at sites of leukocytoclastic lesions makes PTX3 a key factor for the induction of leukocytoclasia in small-vessel vasculitis.

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Fever-like Temperatures Inhibit NF- κ B-Dependent Anti-apoptotic Signals by Dissociation of HSP90 from the IKK Complex

R. Kettritz, B. Lauterbach, M. Choi, S. Rolle, M. Wellner, C. Scheidereit, F.C. Luft

Clinic, Medical Faculty of the Charite, Humboldt University of Berlin, Germany

Neutrophil apoptosis is implicated in ANCA-mediated cytokine-rich inflammation. Whereas decreasing ANCA-induced neutrophil activation by apoptosis may be beneficial, increasing ANCA target antigen expression on apoptotic neutrophils may be deleterious. Infections can complicate ANCA-disease. We tested the hypothesis that short-term exposure to fever-like temperatures modifies neutrophil apoptosis. We found that heat exposure abrogated delayed apoptosis by TNF- α and LPS. In contrast, constitutive apoptosis and delayed apoptosis by GM-CSF and IL-8 were not affected. By FACS, we demonstrate that this inhibitory effect did not result from temperature-mediated shedding of cytokine receptors. Studying anti-apoptotic pathways, we observed that TNF- α and LPS, but not GM-CSF and IL-8, activated NF- κ B. However, short-term heat exposure inhibited NF- κ B activation. Moreover, heat exposure as well as specific inhibition of the IKK complex by a small peptide consisting of the NEMO binding domain (NBD) with an HIV-TAT protein transduction domain, prevented delayed apoptosis by TNF- α and LPS. In addition to the effects on apoptosis, heat exposure blocked NF- κ B dependent gene transcription as demonstrated by quantitative RT-PCR. Finally, we observed that heat exposure affected the composition of the IKK complex. HSP90, which is required for the IKK activity, dissociated from the complex after short-term heat exposure. Our study suggests that fever-like temperatures can prevent anti-apoptotic signals resulting in increased apoptosis of circulating neutrophils. This effect may be important during fever that may complicate the course of ANCA-related disease.

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Microscopic Polyangitis in the Setting of Usual Interstitial Pneumonia

G. Gregorini, F. Londrino, G. Battaglia, S. Monti, A. Tironi, U. Specks, K. Keogh

Mayo Clinic Rochester, Pulmonary and Critical Care Medicine, Rochester, USA

Objectives: A relationship between ANCA-associated vasculitis and pulmonary fibrosis has been proposed through recent case reports. The aim of this dual-center observational cohort study is to characterize a patient population with co-existent diagnoses of Usual Interstitial Pneumonia (UIP) and Microscopic Polyangiitis (MPA).

Methods: Patients from both centers were identified by directed search strategies of clinical databases. Patients were included when they fulfilled criteria for the diagnosis of both UIP and MPA.

Results: A total of 32 patients meeting these criteria were identified, (55% male, 16 from each center) with a mean age of 67 ± 10

years. The clinical characteristics of the US and Italian cohorts were similar and so data was pooled for analysis. All patients were P-ANCA/MPO positive and no patient was C-ANCA/PR-3 positive. 72% had clinical evidence of glomerulonephritis, 35% had nervous system involvement and 16% had dermatological vasculitis. All 32 patients had radiographic evidence of UIP. In review of computed tomography of the chest, available in 31/32, 72% had basilar predominant interstitial infiltrates, 91% had peripheral honeycombing, and 72% had traction bronchiectasis – features characteristic of UIP. 34% also had ground glass infiltrates, suggestive of inflammation. Pulmonary function testing at last available visit was consistent with UIP with a mean diffusing capacity of carbon monoxide $50.8 \pm 15\%$ and a total lung capacity of $73.7 \pm 15\%$. In the majority of patients (65%) both diagnoses were made during the same episode of care. In 19% UIP preceded and in 16% MPA was the preceding diagnosis.

Conclusion: Here we describe a large cohort of patients with UIP and P-ANCA/MPO-associated vasculitis. The clinical picture of the vasculitis is that of MPA. The diagnosis of UIP was generally made concurrent with or preceding the diagnosis of vasculitis, suggesting that the fibrotic lung changes precede the onset of MPA.

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Epidemiologic Study of Environmental Exposures and Development of ANCA-Associated Small Vessel Vasculitis with Glomerular Involvement

S.L. Hogan, G.S. Cooper, L.A.N. French, A.J. Imler, C.G. Parks, D.A. Savitz, H. Chin, C.E. Jennette, J.C. Jennette, R.J. Falk

University of North Carolina, Nephrology & Hypertension, Chapel Hill, USA

The purpose of this study was to evaluate the association of silica, metals, solvents and pesticides with ANCA-associated small vessel vasculitis (ANCA-SVV) in a population-based (southeastern U.S.) case-control study. All cases had biopsy-proven glomerular involvement of ANCA-SVV. Controls were frequency matched to cases by gender, age and state. Lifetime occupations and specific tasks were assessed by structured telephone interviews in 131 cases and 109 controls. Exposures were assessed by industrial hygienists (LAN-F, AI) and an epidemiologist (CGP). Cumulative exposure in years (yrs) was ranked and evaluated across tertiles. Pesticide exposure was only assessed as ever/never. Logistic regression controlling for age, gender and state was used to evaluate the association of each exposure with ANCA-SVV. Adjusted odds ratios (Adj OR) and 95% confidence intervals (CI) are presented. Cases were 60% male, 84% Caucasian and had a median age of 63 yrs. Ever versus (vs.) never exposed, ranked duration and highest tertile of exposure duration for metals and solvents were not associated with ANCA-SVV (adj ORs from 0.97 and 1.08). There was a trend for associations between the highest tertile of silica exposure duration and pesticide exposure (ever vs. never) with ANCA-SVV. This study suggests increased duration of silica exposure and pesticide exposure (ever vs. never) may be associated with the onset of ANCA-SVV, but provides evidence against an association based on the other indices considered.

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Preliminary Investigation of Heavy Metal Exposure in Patients with Wegener's Granulomatosis

D. Albert, F. Barnack, M. Zlupko

University of Pennsylvania, Medicine/Rheumatology, Philadelphia, PA, USA

Introduction: Previous studies (Arthritis Care and Research Vol. 51, p. 656, 2004 and Journal of Clinical Rheumatology, in press) have suggested several environmental exposures that might be related to Wegener's Granulomatosis including mercury and lead. In this study we extended our findings to measurement of heavy metals in blood samples from patients with Wegener's compared with patients with gout and osteoarthritis.

Methods: Using commercial laboratory testing, patients obtained whole blood heavy metal screening that was compared with questionnaire estimates of excessive exposure.

Results: Thus far we have enrolled 9 patients with Wegener's, 4 patients with gout and 3 patients with osteoarthritis. The results of the blood analyses and questionnaire estimates are shown below. 3 of 9 Wegener's patients, 3 of 4 gout patients and 2 of 3 osteoarthritis patients had abnormal laboratory values. In Wegener's, 2 of 3 had elevated nickel and 2 of 3 had elevated cobalt whereas 1 of 3 had elevated mercury. In gout, 2 of 3 had elevated cobalt, and 1 of 3 had elevated copper. In osteoarthritis, 1 of 2 had elevated cadmium and 1 had elevated cobalt.

Summary: The questionnaire accurately identified the elevated mercury, and copper and 1 each with nickel and cadmium. Cobalt elevations in 3 patients were not identified by the questionnaire, nor was one patient with elevated cadmium. Overall, the questionnaire was only 50% sensitive and 52% specific for detectable elevations in whole blood heavy metal testing.

Conclusions: From these preliminary studies it appears that elevated cobalt is a common finding that does not track with Wegener's but nickel and mercury could. Further analysis with a larger data set and more sensitive testing such as hair or nail analysis might yield improved results.

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Comparison of Disease Activity Measures for ANCA-Associated Vasculitis

P.A. Merkel, D. Cuthbertson, B. Hellmich, G.S. Hoffman, D.R.W. Jayne, C.G.M. Kallenberg, J. Krischer, R. Luqmani, A. Mahr, E.L. Matteson, U. Specks, J.H. Stone, Vasculitis Clinical Research Consortium

Boston University School of Medicine, Rheumatology, Boston, MA, USA

Purpose: There are currently several different instruments used to measure disease activity and extent in clinical trials of ANCA-associated vasculitis leading to division among investigative groups and difficulty comparing study results. As part of an effort to develop a new international consensus and data-driven disease assessment tool, we performed an exercise comparing 7 different vasculitis instruments.

Methods: Ten experienced vasculitis investigators from 5 countries in the USA (5) and EU (5) scored 20 paper cases of Wegener's granulomatosis or microscopic polyangiitis using 7 different disease assessment tools: 5 activity measures: BVAS1, BVAS/WG, BVAS2003, a physician global assessment (PGA), Disease Extent Index (DEI); a persistent disease measure: BVAS2; a prognostic tool: Five Factor Score (FFS). Cases were drafted based on actual cases from 4 centers, including Rheumatology, Nephrology, and Pulmonology clinics. 5 cases were re-scored by all raters 1–2 weeks after initial exercise.

Results: Reliability of the measures was extremely high with ICCs for the 7 measures ranging from 0.89–0.98. Within each instrument, there were no significant differences or outliers among the 10 investigators' scores. Test/re-test reliability was also high for each of the measures, ranging from 0.76–0.95. The FFS and BVAS2 both correlated less well with the activity measures. There was no significant difference between the scores of the 5 US investigators compared to the 5 EU investigators. The scores of the 5 activity measures correlated extremely well with one another as outlined in the Table.

Conclusions: The currently available tools for measuring disease extent and activity in ANCA-associated vasculitis are highly correlated and reliable. However, important differences exist in scoring methodology, weighting, disease status definitions, and in application of the tools in clinical trial design and interpretation. The data and insight gained from this exercise provide us with confidence as we go forward with developing, based on existing tools, an improved and universally accepted disease assessment instrument.

Table Correlations Among Vasculitis Activity Scores. All p-values <0.001.

	DEI	PGA	BVAS2003	BVAS/WG
BVAS1	0.78	0.88	0.94	0.86
BVAS/WG	0.83	0.83	0.89	
BVAS2003	0.83	0.86		
PGA	0.75			

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Deoxyspergualin in Relapsing Wegener's Granulomatosis

O. Floßmann, D.R.W. Jayne

Addenbrookes Hospital, Nephrology, Cambridge, United Kingdom

Introduction: Wegener's granulomatosis (WG) is a chronic relapsing form of small vessel vasculitis. Although therapy with cyclophosphamide and corticosteroids leads to remission in over 90% of patients, relapse is common. Current therapy is associated with significant long term complications.

Methods: This multi-centre, single limb, open-label study investigated a novel immunosuppressant, deoxyspergualin (DSG), in patients with relapse of WG after they received standard remission induction therapy with cyclophosphamide or methotrexate and corticosteroids for at least 3 months and being on maintenance therapy with either azathioprine or mycophenolate mofetil. Inclusion required a Birmingham Vasculitis Activity Score (BVAS) of at least 5. DSG was self-administered subcutaneously at a dose of 0.5 mg/kg/day. It was given in 6 treatment cycles of up to 21 days. The cycle was stopped when the WBC fell below 4,000/ml with a washout period of at least 7 days before the start of the next cycle. The endpoint was complete remission (BVAS = 0 for at least 2 months) or partial remission (BVAS < 50% of entry). We are presenting interim results from our centre.

Results: 13 patients have completed at least 3 months of the treatment phase. 13/13 patients achieved at least partial remission and 6/13 complete remission. The BVAS fell from a mean $\pm$ SE of 12.78 ± 1.92 at study entry to 0.89 ± 0.45 at the end of the study. The mean $\pm$ SE dose of prednisolone was 17.32 ± 1.17 in the first month, it was 7.5 ± 0.74 in the sixth. There were 2 hospital admissions in 1 patient in the first 2 treatment cycles which were due to active vasculitis. Although there were several cases of various infections, none required hospital admission. 1 patient was diagnosed with Pneumocystis pneumonia 3 months after the end of the study. Discussion: DSG appears to be effective in inducing remission in patients with relapsing WG. No severe adverse events were seen during the DSG treatment period.

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Effective Treatment of Refractory Vasculitis by B Cell Depletion with Rituximab

K. Smith, S. Burns, D.R.W. Jayne

Addenbrooke's NHS Trust, Dept of Renal Medicine, Cambridge, United Kingdom

In a prospective study, 12 patients with refractory or relapsing vasculitis were treated with Rituximab and followed for a mean of 15 months. Diagnoses were: Wegener's granulomatosis 5, microscopic polyangiitis 5 and Churg Strauss angiitis 2. Patients received four, weekly doses of Rituximab 375mg/m² and one pulse of cyclophosphamide 500mg. All patients achieved complete depletion of circulating B cells for at least five months. 10 achieved remission of all features of active vasculitis, one had a partial remission and one had no response. Clinical response was reflected by significant falls in C-reactive protein, ANCA and daily prednisolone requirement. Relapses were seen in five patients after a mean of 11 months and occurred either at the time or subsequent to B cell reconstitution. Of four re-treated with Rituximab, all returned to remission, although one relapsed for a second time after a further six months. No serious adverse events were seen moderate infusion reactions occurred in three and one infection was seen, no change in total IgG levels occurred. Rituximab appears to be a safe effective therapy for vasculitis capable of inducing sustained remission.

Autologous Stem Cell Transplantation in Systemic Vasculitis – A Single Center Experience, Summary of the EBMT Database and Review of the Literature

I. Kötter, T. Daikeler, C. Amberger, C. Bocelli-Tyndall, L. Kanz, A. Tyndall, I. Kötter, T. Daikeler, C. Amberger, C. Bocelli-Tyndall, L. Kanz, A. Tyndall

University Hospital, Internal Medicine II, Tübingen, Germany

Introduction: Despite intensive immunosuppressive or cytotoxic therapy the systemic vasculitides may be refractory to treatment and be life threatening. In general, cyclophosphamide (CY) is used to induce remission. However, its use over a long period of time is limited due to infections and secondary malignancy. We report on 4 patients who received an autologous stem cell transplantation (ASCT) for treatment resistant systemic vasculitis. Cases: Patient 1: A 33 year old female Wegener's granulomatosis with ENT and renal involvement had received a n HLA matched live kidney transplant from her sister. Despite the use of cyclosporin A (CSA) she needed further oral treatment with CY (100 mg/day) because of relapsing pulmonary infiltrations. After a cumulative dose of 100g CY and increasing disease activity she underwent ASCT after conditioning with CY 200 mg/kg bw and ATG 20 mg/kg bw. Five months later she had to be treated against EBV reactivation, to date (six years after ASCT) she is in complete remission under host versus graft protection with mycophenolate mofetil (MMF). Patient 2: a 36 year old female with relapsing polychondritis affecting ears, ribs, cornea and thoracic aorta after inefficacy of etanercept, anakinra, CSA, MMF, leflunomide, CY, fludarabine, sirolimus and steroids in various combinations. She received ASCT with CY 200 mg/kg bw and ATG 20 mg/kg bw and is in complete remission 5 months after the procedure with a daily prednisolone dose of 7.5 mg. Patient 3: a 30 year old female with Churg Strauss syndrome and cerebral vasculitis, asthma and glomerulonephritis received ASCT with 200 mg Cy/kg bw only, due to massive allergic reaction to ATG. Her previously ineffective treatments consisted of azathioprine, CY, CSA, MTX, steroids in various combinations. She also had received Interferon alpha, which had maintained remission but induced a progressive nephritic syndrome. Nine months after ASCT she is in complete remission under CSA. She had to be treated with acyclovir because of herpes simplex reactivation 5 months after ASCT. Patient 4: a 21 year old female with severe Takayasu arteritis underwent ASCT after inefficacy of MTX, CY, MMF, azathioprine, CSA, infliximab and anakinra. One month after ASCT she had a CMV reactivation, 7 months after ASCT she had a mild relapse of TA and is effectively treated with prednisolone and azathioprine.

Discussion and Conclusions: ASCT is effective in treatment resistant vasculitis. It may not be curative, but relapses are markedly less severe and appear to respond better to conventional treatment after ASCT. ASCT was well tolerated, but infections were common, especially reactivation of viruses from the herpes family (EBV, CMV,

HSV). ASCT with in vivo and in vitro purging (stem cells were CD34+ -selected) leads to substational immunosuppression comparable to that occurring in allogeneous transplantations. An intensive prophylaxis for infections (antibiotics, antimycotics, virustatics) is recommended. According to the EBMT database, in addition to the 4 patients presented here, 21 patients with vasculitis – 8 with cryoglobulinemia, 6 with Behcet's disease, 3 with unclassified systemic vasculitis, 2 with PAN and 3 with WG who underwent ASCT were reported. Follow up data and a review of the current literature will be presented.

Microarray Expression Analysis of Systemic Lupus Erythematosus and Anti-neutrophilic Autoantibody (ANCA) Vasculitis Patient Leukocytes Reveals Disease Specific Gene Expression Signatures

A. David, D.A. Barnes, M.A. Dooley, P Sullivan, B. Jonas, R. Thomas, H. Chen, C. Candiani, J.C. Jennette, R.J. Falk

University of North Carolina, Medicine-Hypertension/Nephrology, Chapel Hill, USA

Objective: Leukocytes in patients with autoimmune inflammatory diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and ANCA vasculitis play a major role in development and progression of disease. Our goal is to identify alterations in leukocyte gene expression between healthy and affected individuals that may reveal disease related processes and suggest potential pathways for clinical intervention.

Methods: Blood leukocyte RNA was isolated from 72 samples from 45 SLE, 25 samples from 21 ANCA vasculitis patients, 26 samples from different RA patients and 28 samples from different healthy donors. Following hybridization to microarrays, Genesis (Gene Logic) and AVADIS (Strand Genomics) bioinformatic software packages identified differentially expressed gene sets.

Results: Five hundred and forty-one differentially expressed gene fragments from blood leukocytes revealed signatures that differentiated RA, ANCA, SLE and healthy donor populations. A core of interferon-inducible gene fragments made up the SLE signature, were activated in healthy donor CD4+ T-lymphocytes and a subset was elevated in SLE kidney biopsy RNA. ANCA patients expressed a gene signature, predominantly expressed in neutrophils. Comparison of individual signature-gene expression levels with clinical activity measures showed a strong correlation of many ANCA genes with BVAS ($R \sim 0.7$) but a much lower correlation for SLE genes with BILAG or SLEDAI ($R < 0.4$). No RA specific signature was found.

Conclusion: Leukocyte gene expression data is capable of differentiating patients with different autoimmune diseases. In the future, a focused gene array may aid clinicians in differentiating diseases, in monitoring disease activity and therapeutic response, or even in predicting therapeutic effectiveness.

Interferon-alpha for Refractory Churg-Strauss Syndrome. First Results of a Phase II Prospective Open Label Study

C. Metzler, W.L. Gross, B. Hellmich

Universitätsklinikum Schleswig-Holstein, Med.
Krankenhausabteilung, Rheumaklinik Bad Bramstedt,
Germany

Background: We previously reported the successful induction of remission by the use of Interferon-alpha (IFN-alpha) in four patients with severe Churg-Strauss Syndrome (CSS) who were refractory to standard treatment with corticosteroids (CS) and cyclophosphamide (CYC) or methotrexate (MTX) [1].

Objective: To examine prospectively the safety and efficacy of interferon-alpha (IFN-alpha) for induction of remission in a larger cohort Churg-Strauss Syndrome (CSS) with contraindications to other immunosuppressive agents.

Patients and Methods: In a phase II prospective open label study, 8 patients with active CSS despite treatment with CS plus CYC or MTX received recombinant human IFN-alpha 2b 3 Mio. I.U 3 times weekly s.c. for induction of remission. Primary end point was the successful induction of remission. Secondary end points were the dosage of concomitant CS and side effects.

Results: Among the eight patients, treatment with IFN-alpha induced complete remission in 5 and partial remission in the three other patients after a mean time of 3 (+/- 1.6) months. The mean DEI score fell significantly from 4.2 (+/- 2.4) to 1.5 (+/- 1.4) ($p = 0.045$) and the mean BVAS showed a significant decrease from 7.0 (+/- 3.0) to 0.3 (± 0.5) ($p = 0.004$). The eosinophil count in peripheral blood also decreased from 1715.8/ μ l (+/- 1818.0) to 249.8/ μ l (+/- 172.6) ($p = 0.143$). At the time of remission, mean dosage of IFN-alpha was 9 (+/- 2.8) million I.U./week s.c. It was possible to taper concomitant prednisolone from a mean dose of 20.8 mg/d (+/- 13.6) to 5.7 mg/d (+/- 2.0) ($p = 0.207$) after a mean time of 3 (+/- 1.6) months. During this period, only malaise on the day of injection was reported by one patient, no severe side effects were observed.

Conclusion: IFN-alpha is a safe and effective treatment for induction of remission in patients with severe CSS being refractory to conventional immunosuppressive treatment. Further clinical trials are needed to examine the efficacy of INF-alpha treatment for maintenance of remission in CSS.

Reference

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Membrane Proteinase 3 Expression in Wegener's Granulomatosis Patients and in Human Hematopoietic Stem Cell-derived Neutrophils

A. Schreiber, B. Otto, X. Ju, M. Zenke, U. Goebel,
F.C. Luft, R. Kettritz

Franz-Volhard-Klinik, Nephrology & Hypertensiology,
Berlin, Germany

A large membrane PR3 (mPR3)-positive neutrophil subset (mPR3high) is a risk factor for Wegener's granulomatosis (WG). We investigated the relationship between mPR3 expression and clinical manifestations in 81 WG patients and studied mPR3 expression in CD34+ stem cell-derived human neutrophils. The mPR3 high neutrophil percentage correlated with renal function, anemia, and albumin at the time of presentation. The mPR3high neutrophil percentage and renal failure severity correlated directly after 5 years. To elucidate mechanisms governing mPR3 expression, studies were conducted to determine whether or not the genetic information governing mPR3 expression resides within the neutrophils. CD34+ hematopoietic stem cells were differentiated to neutrophils and their mPR3 expression determined. With differentiation, cells progressively expressed neutrophil surface markers and demonstrated a strong respiratory burst. Intracellular PR3 was detectable from day 4 by Western blotting. An increasing percentage of an mPR3-positive neutrophil subset became detectable by FACS while a second subset remained negative, consistent with a bimodal expression. Finally, human PR3-ANCA induced a stronger respiratory burst, compared to human control IgG in stem cell-derived neutrophils. Our studies underscore the clinical importance of the WG mPR3 phenotype. The surface mPR3 on resting cells is probably genetically determined rather than being dictated by external factors.

Increased Membrane Expression and Plasma Concentrations of Proteinase 3 (PR3) in Vasculitis is not a Consequence of the -564 A/G Gene Promotor Polymorphism

M. Abd-Gawad, T. Hellmark, L. Gunnarsson,
K. Westman, M. Segelmark

Lund University, Nephrology, Lund, Sweden

Background: Beside being the major autoantigen there are several reported findings linking PR3 to small vessel vasculitis (i) increased circulating levels of the enzyme in patients even during stable remission; (ii) increased levels of membrane-PR3 (mPR3) on resting neutrophils and (iii) skewed distribution of a polymorphism in the promotor region of the PR3 gene generating a potential Sp 1 site. Here we elucidate how these findings relate to each other.

Methods: We measured plasma concentrations of PR3 by sandwich ELISA using affinity purified rabbit-antihuman-PR3 and mouse monoclonal 4A3 and 4A5, mPR3 expression by FACS using 4A5 and the gene polymorphism -564 A/G by real-time PCR. We compared

results from 63 patients with ANCA associated vasculitis (most of them in stable remission) with 107 healthy blood donors.

Results: As previously reported AASV patients had increased plasma concentrations of PR3 (median 192 ug/l vs 148 ug/l), increased number of PR3-positive resting neutrophils (66.4% vs. 44.1%, $p < 0.001$) and increased fluorescence index. However, contrary to a previous report we found no skewed distribution of the PR3 gene. The G allele, which is presumed to mediate high PR3 transcription, was found in homozygote form in 44% and in heterozygote form together with the A allele in 40% of the patients compared to 46% and 44% respectively among the controls (not significant). There was a weak correlation between mPR3 and plasma PR3 among MPO-ANCA patients ($r = 0.433$, $p = 0.039$), but neither measurement showed any statistical correlation with the different gene alleles.

Conclusion: Increased plasma PR3 and mPR3 in AASV is not a consequence of the -564 A/G polymorphism in the PR3 promoter. It is possible that the increased plasma levels and the increased mPR3 levels have a common origin, which might be a fundamental predisposing factor for the generation of the anti-PR3 autoantibodies (=PR3-ANCA).

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Wegener's Autoantigen Induces Maturation of Dendritic Cells and Licences them for Th1 Priming via the Protease-activated Receptor-2 Pathway

M. Ai, W.L. Gross, A. Müller, B. Hellmich, P. Lamprecht, E. Csernok

University of Schleswig-Holstein, Campus of Lübeck, Rheumatology and Rheumaklinik of Bad Bramstedt, Bad Bramstedt, Germany

Objective: To address the question how a particular autoantigen – proteinase 3 (PR3) (Wegener's autoantigen) – becomes target of adaptive immunity, we investigated the effect of PR3 on the maturation of dendritic cells (DCs) and by which mechanism this effect was induced.

Methods: Immature DCs (iDC) were generated from human peripheral blood monocytes and stimulated with PR3, human leukocyte elastase and tumor necrosis factor alpha. The maturation phenotype of DCs (mDC) was analysed by flow cytometry. PR3-specific T cell priming by dendritic cells was examined by using a cytometric cytokine secretion assay (IFN-alpha/IL-4 capture assay). The expression of protease-activated receptor-2 (PAR-2) was investigated by flow cytometry, real-time-PCR and by immunohistochemical analysis.

Results: We found that PR3 induces phenotypic and functional maturation of blood monocyte-derived iDCs. PR3-treated DCs express high levels of CD83 (CD83 positive iDC 3% vs mDC 31% $P = 0.007$), a DC-restricted marker of maturation, the costimulatory molecules (CD80 positive iDC 60% vs mDC 89%, $P = 0.035$, and CD86 positive iDC 19% vs mDC 45%, $P = 0.020$), and major histocompatibility complex type II (MHC-II positive: iDC 57% vs mDC 99%, $P = 0.049$). Subsequent to PAR-2 mediated maturation, DC become fully competent antigen presenting cells and can therefore induce stimulation of PR3-specific CD4+ T cells, which produce INF-alpha; and drive the polarization towards the Th1-type phenotype typical of WG. We have demonstrated that PR3 activates iDCs via the

PAR-2, a G protein-coupled receptor known to trigger inflammatory responses in vivo. The maturation of iDC mediated through PR3 was inhibited by a serine protease inhibitor (PMSF), by antibodies directed against PAR-2 and by inhibition of phospholipase C, thus confirming the involvement of PAR-2.

Conclusion: Thus, Wegener's autoantigen interacts with a 'gateway' receptor (PAR-2) on iDCs in vitro triggering their maturation and licences them for a Th1 response that is critical for the generation of granulomas in WG. In conclusion, our study provides first evidence that the autoantigen (PR3) participates in the initiation of an adaptive response that finally may lead to the production of PR3-ANCA.

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Surface-bound Elastase on Polymorphonuclear Neutrophils (PMN): Identification of CD11b as the Ligand

C. Wagner, K. Andrassy, G.M. Hänsch

Institut für Immunologie, Immunology, Heidelberg, Germany

Elastase is a major serine protease of polymorphonuclear neutrophils (PMN). Upon activation of PMN, the preformed protein is mobilized from intracellular stores and, depending on the activating conditions, is either released into the supernatant, or, alternatively, is bound to the cell surface. By a variety of methods, including uptake -, and crosslink-studies, as well as confocal laser scan microscopy, we now provide evidence, that elastase binds to the $\beta 2$ -integrin CD11b. There, a conformational alteration of CD11b is induced, apparent as expression of a neodeterminant. Expression of elastase on PMN is also observed in patients with active Wegener's granulomatosis, and on PMN of patients with Staphylococcus aureus-induced localized infections as well. Similarly to the in vitro data, elastase surface expression and conformational alterations of CD11b were seen, particularly on the PMN recovered from the infected site. The presence of elastase at the site of inflammation is in keeping with its presumed role in leukocyte trafficking and host defense. On the other hand, due to its potential for degrading extracellular matrix proteins, elastase could participate in localized tissue damage as it occurs in severe S.aureus infection.

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The ANCA-activated Neutrophil Respiratory Burst is Inhibited by Adenosine in the Presence of Endothelial Cells (EC) While Neutrophil Degranulation Persists and Mediates vWf Release

X. Lu, G. Nash, C.O.S. Savage

The School of Medicine, University of Birmingham, Birmingham, B15 2TT

Objectives: To determine the mediators of EC injury in ANCA-Associated Systemic Vasculitis (AASV), we investigated the factors

controlling the neutrophil respiratory burst and endothelial vWf release during neutrophil-EC co-cultures.

Methods: Superoxide release during static neutrophil-EC co-culture, or from neutrophils binding to P-selectin adhesion molecules or TNF-activated EC under conditions of flow, was measured using the superoxide dismutase inhibitable reduction of ferricytochrome. Neutrophils were activated with fMLP (1 μ M), normal IgG or ANCA-IgG (200 μ g/ml). vWf was measured by ELISA. Serine protease activity was measured enzymatically.

Results: fMLP or ANCA-IgG induced superoxide release from neutrophils binding to P-selectin but not those binding to EC. In static assays, EC inhibited superoxide production. Adenosine inhibited the respiratory burst and in co-cultures adenosine deaminase overcame the inhibitory effects of EC. Serine proteases were released during activated neutrophil-EC co-culture. There was enhanced release of vWf from during co-culture which was not inhibited by diphenyleneiodonium nor by superoxide dismutase plus catalase but was inhibited by diisopropylfluorophosphate.

Conclusions: EC inhibit superoxide generation by FMLP and ANCA-activated neutrophils. vWf release occurs during co-culture and is sensitive to serine protease but not NADPH oxidase inhibition. Serine proteases may be more important than reactive oxygen species as mediators of endothelial injury during AASV.

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Myeloperoxidase Binds Endothelial Cytokeratin 1 and Interferes with the Production of the Vasoregulatory Peptide Bradykinin

J. Astern, W.F. Pendergraft III, J.C. Jennette, R.J. Falk, G.A. Preston

University of North Carolina, Chapel Hill, USA

Myeloperoxidase (MPO) is an abundant neutrophil granule constituent that when released into circulation is internalized by endothelial cells and interferes with normal vascular function. We hypothesized that internalization of MPO by endothelial cells is a receptor-mediated event. Coimmunoprecipitation and immunofluorescent colocalization experiments identified cytokeratin 1 (CK1) as a putative endothelial MPO receptor. Because CK1 is a member of an endothelial receptor complex for the plasma kallikrein-kininogen system (KKS) that produces the potent vasoactive peptide bradykinin, we hypothesized that MPO would interact with and modulate the KKS, thus interfering with bradykinin production and altering vascular function. Circulating high molecular weight kininogen (HK) and prekallikrein complexes bind endothelial CK1, after which prekallikrein is activated to cleave bradykinin from HK. We expected that MPO would compete with kininogen for endothelial CK1 binding sites; however, our HUVEC-based binding studies revealed a 5-fold increase in HUVEC-bound biotinylated HK (bHK) in the presence of a 50-fold molar excess of MPO. In vitro protein binding assays showed that MPO and kininogen directly interact, which may account for the increased detection of bHK. The MPO-HK interaction sterically hindered kallikrein's cleavage of bradykinin from kininogen in a cell-free system as analyzed by bradykinin ELISA. Additionally, hypochlorous acid (HOCl), a powerful oxidant generated by MPO that modifies and

alters the function of several proteins, inactivated 50 nM kallikrein at doses as low as 6.25 μ M. Bradykinin cleavage by kallikrein from HOCl-pretreated kininogen was inhibited in a cell-free system, as analyzed by bradykinin ELISA. Thus, CK1 is a possible receptor for MPO that may mediate its internalization. Additionally, MPO and its associated oxidants modulate bradykinin production revealing new vasoregulatory mechanisms associated with the innate immune system.

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Generation and Characterization of Monoclonal Antibodies Specific for Murine Myeloperoxidase

D. Huugen, A. van Esch, H. Xiao, J.C. Jennette, J.W.C. Tervaert, P. Heeringa

University of Maastricht, Clinical and Experimental Immunology, Maastricht, Netherlands

Necrotizing crescentic glomerulonephritis (NCGN) is associated with antineutrophil cytoplasmic autoantibodies (ANCA) either directed against myeloperoxidase (MPO) or proteinase 3 (PR3). Passive transfer of anti-MPO IgG from murine MPO (mMPO)-immunized MPO-knockout mice into wild-type (WT) mice induces NCGN and vasculitis. We report the generation and characterization of a panel of anti-mMPO monoclonal antibodies (mAbs).

Following standard procedures, spleen cells from mMPO-immunized MPO-knockout mice were used to generate mMPO-specific hybridomas. Reactivity with mouse, rat, and human MPO was determined by immunohistochemistry and ELISA. Differential epitope recognition was tested by inhibition ELISA. Pathogenicity of IgG1 mAbs was tested in the context of a systemic proinflammatory stimulus in a dose that aggravates polyclonal anti-mMPO IgG-induced NCGN (bacterial lipopolysaccharide, LPS, 0.5 μ g/g). Eleven different mMPO-recognizing mAbs were generated (see table). Inhibition ELISA revealed that all IgG1 mAbs bound to one of three epitopes on native mMPO. Specificity for mMPO was determined by immunohistochemistry using WT and MPO-knockout spleen sections. All IgG1 mAbs except 3F7 and 4B3 cross-reacted with rat MPO on immunohistochemistry, and only one cross-reacted with

Table

Clone	Cross-inhibited by	Isotype	Cross-reactivity with rat MPO	Cross-reactivity with human MPO
8F4	4H9	IgG1	+	—
2D4	8F4, 4H9, 6G4	IgG1	+++	—
4H9	8F4, 6G4	IgG1	++	+
1F1	3B1, 1H4, 6G4	IgG1	+	—
1H4	3B1, 1F1, 6G4	IgG1	+	—
3B1	1H4, 1F1, 3F7 6G4	IgG1	+	—
3F7	4B3, 6G4	IgG1	—	—
4B3	3F7, 6G4	IgG1	—	—
6G4	4H9, 1F1, 3B1, 1H4, 8F11	IgG2a	ND	ND
8F11	ND	IgG2a	ND	ND
6D1	ND	IgG2b	ND	ND

human MPO. Alone or in several combinations, IgG1 anti-mMPO mAbs +0.5 microg/g LPS induced minor histopathological and urinary alterations, but no full-blown NCGN, after sacrifice on day 6.

In conclusion, we generated a set of anti-mMPO mAbs with different isotypes, allowing us to study the role of different isotypes and epitopes in the pathogenesis of anti-mMPO IgG-induced NCGN. Furthermore, anti-mMPO IgG1 mAbs with LPS do not cause NCGN. We are currently extending these studies, investigating the pathogenicity of anti-mMPO IgG2a and IgG2b mAbs in the context of a systemic proinflammatory stimulus (LPS) or a subnephritogenic dose of anti-GBM antibodies.

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Influenza Vaccination in 230 Patients with ANCA-associated Vasculitis: No Evidence for an Increase in Relapses

P. Stassen, J.-S. Sanders, C.G.M. Kallenberg, C.A. Stegeman
UMCG, Internal Medicine, Groningen, The Netherlands

Background: Case-reports and series describing a relation between influenza or other vaccinations and activity of autoimmune diseases such as vasculitis suggest a causal role. Larger studies have failed to confirm this. In practice, many patients with vasculitis are advised against influenza vaccination, despite an increased risk for influenza induced morbidity and mortality. We examined the relation between influenza vaccination and relapse of ANCA-associated vasculitis (AAV).

Methods: We registered influenza vaccination status and relapses in all patients with AAV followed for at least 1 year, in the period October 1999 to October 2004. As influenza vaccinations are performed in October, a patient was considered vaccinated or not for every 12 month period following this month. Relapse rates per 100 patients at risk according to vaccination status were calculated for 3 month periods. Results: 230 patients were included (WG n = 172; MPA n = 25; RLV n = 15; CSS n = 18). Mean follow-up was 48

Figure

(range 13–60) months. Per year 57.6–62.4% of patients were vaccinated. Vaccination status was not associated with previous relapses or disease duration. 165 relapses occurred during the study period. In patients vaccinated relapse rate was 3.4 per 3 months/100 patients at risk, compared to 6.2 for patients not vaccinated. Also when 3 months periods were compared, patients vaccinated had lower relapse rates than patients not vaccinated (figure).

Conclusion: We found no increase of relapses of AAV after influenza vaccination. Although we could not demonstrate it, bias by indication may have influenced our results. Until further prospective data become available, it seems safe to vaccinate patients with AAV against influenza.

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Risk Factors for Herpes Zoster in Immunocompromised Patients: Experience from the Wegener's Granulomatosis Etanercept Trial

P.K. Wung, J.T. Holbrook, G.S. Hoffman, A.K. Tibbs, U. Specks, Y.-I. Min, P.A. Merkel, R. Spiera, J.C. Davis, E.W. St. Clair, J. McCune, S.R. Ytterberg, N.B. Allen, J. Stone
Johns Hopkins University, Rheumatology, Baltimore, USA

Purpose: To evaluate the risk factors for herpes zoster (HZ) as well as the incidence and timing of this complication in patients who were treated with immunosuppression because of active Wegener's granulomatosis (WG).

Subjects and Methods: We studied the 180 WG patients in the Wegener's Granulomatosis Etanercept Trial (WGET) cohort, comparing the HZ incidence rate among patients who had experienced HZ before trial entry to that of patients with no history of HZ before the trial. HZ events during WGET were documented prospectively. Follow-up questionnaires were employed to describe the location, treatment, and complication(s) of HZ and its therapy. Univariate and multivariate analyses were performed to evaluate risk factors for the occurrence of HZ during the trial. Results: Eighteen patients (10% of the WGET cohort) suffered a total of 19 HZ episodes over a mean follow-up period of 27 months. The annual incidence of HZ in the WGET cohort was 51.7 cases/1,000 patient-years. The median time from enrollment to the occurrence of HZ in the subgroup of patients with that complication was 16.5 months (~9.4). Fifteen of the 19 HZ events (79%) occurred between months 6 and 36, many months after the period of most intensive immunosuppression. In univariate analyses, history of serum creatinine >1.5 mg/dL before enrollment was associated with a relative risk (RR) of 3.0 [95% confidence interval (C.I.): 1.1, 7.8] for HZ during WGET (P = 0.03). In multivariate analyses, serum creatinine >1.5 mg/dL was associated with an RR of 6.3 (95% C.I.: 2.0, 19.8; P = 0.002), and female gender with an RR of 4.7 (95% C.I.: 1.6, 13.2; P = 0.004).

Conclusion: Renal dysfunction and female gender were consistently strong risk factors for HZ events in this population. Contrary to expectation, most HZ events did not occur during periods of most intensive immunosuppression. These data may inform studies of interventions designed to prevent HZ in patients on treatment for immune-mediated diseases.

No Evidence of Parvovirus B19 in Tissue Samples from Patients with Polyarteritis Nodosa and Microscopic Polyangiitis as Assessed by the Polymerase Chain Reaction

Z. Achetto, S.C.B. Costa, P.D. Andrade, R.A. Conde, E.M.I. Amstalden, A.M. Samara, S. Fernandes

UNICAMP, Rheumatology, Campinas, Brazil

Background: Polyarteritis nodosa (PAN) and microscopic polyangiitis (MPA) are rare inflammatory systemic diseases. Infection has been associated with some types of primary systemic vasculitis. Nowadays, human parvovirus B19 (B19) has been implicated as a trigger factor in many inflammatory diseases, including systemic vasculitis. Objective: To investigate whether B19 DNA can be detected in PAN and MPA tissue specimens with vasculitis.

Methods: We evaluated tissue samples obtained from patients with the diagnosis of PAN and MPA according to ACR criteria and Chapel Hill Conference Consensus. All specimens had histopathological features characteristic of vasculitis. The fixed, paraffin-embedded tissue samples obtained were screened for B19 DNA using polymerase chain reaction (PCR) procedures. To confirm the absence of any inhibitor factor, extracted DNA was first amplified with beta-globin-specific primers. The PCR amplification for B19 was performed with a nested-PCR.

Results: Tissue samples obtained from 17 patients with vasculitis had DNA amplified successfully. We confirmed the presence of amplifiable DNA in the tissue samples using PCR primers for beta-globin. Ten patients had the diagnosis of PAN (mean age 29.3 ± 15.1 ; M:F sex ratio 1.5:1) and 7 had MPA (mean age 41.9 ± 18.5 ; M:F sex ratio 1:1.3). The specimens were obtained from skin (11), bowel (3), kidney (2) and muscle, spleen and gallbladder tissue from one patient each. The search of B19 DNA was negative in all the samples evaluated.

Conclusion: Our negative results suggest that B19 unlikely infect arterial tissues of patients with PAN and MPA. We cannot rule out that B19 could

patients with AAV and in 188 healthy controls. Results: The allelic distributions of the PD-1.3 and PD-1.5 SNPs did not differ between patients and healthy controls (HC). Additionally, the allelic distributions of the CTLA4 promoter polymorphisms, -1722T/C, -1661A/G, and -318C/T, as well as the (AT)_n microsatellite in the 3' untranslated region of CTLA4 did not differ significantly between AAV patients and HC. However, the +49G allele was significantly more often present in patients with AAV (70% in patients versus 55% in HC, $P = 0.01$). Furthermore, the co-occurrence of the PD-1.5 T allele with the CTLA4 +49A allele was less often present in patients compared to HC (17% and 32%, respectively, $P = 0.007$).

Conclusions: AAV is associated with the presence of a G allele at the +49 position of CTLA4 and this association is even stronger if also the PD-1.5 C allele is present in the genome. These genetic polymorphisms may lead to hyper-reactivity of T cells and thus may contribute to the pathogenesis of AAV.

No Association between Mannose Binding Lectin Gene Polymorphism and ANCA Associated Vasculitis

L. Karnesh¹, J.M. Heward², J.M. Williams¹, C.O.S. Savage¹, L. Harper¹

¹Division of Immunity and Infection, ²Division of Medical Sciences, Medical School, University of Birmingham, Birmingham, United Kingdom

Patients with ANCA-associated vasculitis (AAV), particularly those with renal involvement, are at high risk of infection following treatment with immunosuppression. Mannose binding lectin (MBL) plays an important role in innate immunity. Genetic polymorphisms of MBL are common and mutations in the MBL gene (A-Wild type, O-Mutant allele designated B, C, D) result in reduced levels of MBL and affects 20% of the population. MBL deficiency in the presence of a compromised immune system is a risk factor for infection and MBL replacement has been suggested for these patients. Low MBL levels may also predispose to the development of autoimmune disease.

Objective: To investigate whether mutant alleles of the MBL gene are associated with ANCA associated vasculitis and a risk for complicating infections.

Methods: MBL alleles were determined by polymerised chain reaction in 92 Caucasians with ANCA positive vasculitis and 188 ethnically matched controls. Results: MBL genotype frequencies were similar for both groups (patient vs control; A/A 71.7% vs 66.4%, A/O 23.9 vs 28.7%, O/O 4.3 vs 4.7%). No association was detected between variant MBL alleles B, C and D and susceptibility to ANCA associated vasculitis ($p = 0.1$). Variant alleles were significantly higher amongst patients who were pANCA positive ($n = 17/26$, $p = 0.027$) but not Wegener's granulomatosis or microscopic polyangiitis. No association was found between MBL genotype and organ involvement or disease severity. MBL status did not affect the incidence of infection or the duration of hospital stay (Incidence of infection per 100 years of follow up: A/A 7.6, A/O 4.9, O/O 7.0).

Conclusion: Our data suggest that MBL polymorphisms are not associated with AAV and do not influence the incidence of concomitant infections. There appears no clinical rationale to replace MBL in patients with ANCA-associated vasculitis and MBL deficiency.

Immunoregulatory Gene Polymorphisms in ANCA-associated Vasculitis

J.W.C. Tervaert, M. Slot, M. Sokolowska, K. Savelkoul, R. Janssen, J. Damoiseaux

University Hospital Maastricht, Department of Clinical and Experimental Immunology, Maastricht, The Netherlands

Background: T cells have been implicated in the pathophysiology of ANCA-associated vasculitis (AAV) because increased T cell activation is present in patients with AAV. T cell activation is regulated by inhibitory molecules such as PD-1 and CTLA-4, whose expression in turn may be affected by gene polymorphisms. We investigated the relationship between polymorphisms in the genes PDCD1 and CTLA4 and the presence of AAV.

Methods: Two single-nucleotide polymorphisms (SNPs) in PDCD1 and five polymorphisms in CTLA4 were investigated in 102

Contribution of CD69 to the Development of Coronary Ateritis Induced with a Vasculitis Inducer *Candida albicans* Water Soluble Fraction

K. Shigeto, K. Suzuki^{1,*}, K. Murayama^{1,2}, T. Nagao^{1,3}, T. Oharaseki^{1,3}, A. Hasegawa⁴, A. Ishida-Okawara¹, N.N. Miura^{1,5}, N. Watanabe⁶, M. Handa⁷, K. Takahashi³, N. Ohno⁵, H. Minamitani⁸, T. Nakayama⁴, T. Arai

¹Department of Bioactive Molecules, National Institute of Infectious Diseases, Toyama 1-23-1, Shinju-ku, Tokyo 162-8640, Japan, ²Department of Applied Biological Science, Science University of Tokyo, ³Department of Pathology, Toho University Ohashi Hospital, ⁴Department of Immunology, Chiba University Graduate School of Medicine, ⁵Laboratory of Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Science, ⁶Department of Internal Medicine, and ⁷Department of Transfusion Medicine & Cell Therapy, Keio University, Graduate School of Medicine, ⁸Graduate School of Science and Technology, Keio University, *Present address: Department of Biochemistry, Weill Medical College of Cornell University, New York

Activated neutrophils are believed to be taken part in the development of vasculitis, because of expression of CD69 on surface of activated neutrophils of patients with rheumatoid arthritis (RA). In addition, injection of activated neutrophils of wild mice into CD69-null mice, which show depression of arthritis induced with anti-type II collagen antibody, allows occurring RA. We here observed depression of incidence of coronary arteritis induced with *Candida albicans* water-soluble fraction (CAWS) 80% in wild mice to 50% in CD69-null mice. Using a multi analysis system for 14 inflammatory cytokines in a single plasma, IFN- α , IL-1 β , IL-3 and RANTES in CD69-null without coronary arteritis significantly were increased, but levels of IL-1 γ , IL-6, IL17 and G-CSF suppressed. MPO-ANCA levels did not show difference between wild and CD69-null mice. In addition, vascular shut-down in CD69-null mice involving thrombus formation showed delay from 5.3 $\pm$ 4.4SD min in wild mice to 18.1 $\pm$ 04.8 SD min. However, platelet aggregation caused by some stimuli in CD69-null mice occurred in the same levels to wild mice in vitro. Moreover, platelet aggregation was caused by activated neutrophils with fMet-Leu-Phe (FMLP) in dose-dependent manner in vitro, suggesting involvement of CD69 expressed on surface of neutrophils in thrombus formation. In deed, we confirmed cellular movement of CD69 of neutrophils when they were stimulated with phorbol ester, FMLP, TNF β and IL-1 γ using imaging analysis by a confocal microscope. Then, platelets bound on adhered neutrophil of wild mouse, but not on neutrophil of CD69-null mouse when neutrophils and platelets were stimulated with FMLP or reactive oxygen species (ROS), indicating that CD69 expressing on neutrophil allowed to be bound with platelets either wild and CD69-null mice. These results show that CD69 contributes to the development of coronary arteritis induced with CAWS through activation of IL-1 γ , IL-6, IL17 and G-CSF, and suppression of IFN- α , IL-1 β , IL-3 and RANTES. Moreover, the expression of CD69 on the surface of neutrophils related with thrombus formation may be involved in the development of coronary arteritis.

Terminally Differentiated Normal Polymorphonuclear Neutrophils Contain Methylated Histones

D. Ciavatta, J.J. Yang, N. Montgomery, T.R. Magnuson, R.J. Falk

Univ. NC at Chapel Hill, Pathology, Chapel Hill, USA

Recently we demonstrated that a hallmark of disease activity in ANCA vasculitis is transcription of primary granule genes. Two of the autoantigens recognized by ANCA, myeloperoxidase (MPO) and proteinase 3 (PR3), were among the genes for which mRNA transcripts were detected in granulocytes of ANCA patients. In normal mature granulocytes MPO and PR3 genes are transcriptionally silent. Gene silencing is associated with covalent modifications of histones, such as methylation of histone H3 at lysine 9 and 27 (H3K9 and H3K27).

Objective: We are interested in determining whether the molecular basis for the difference in gene expression between mature neutrophils from normal individuals and mature neutrophils from ANCA vasculitis patients is, in part, controlled by epigenetic mechanisms.

Method: We isolated polymorphonuclear neutrophils from normal individuals and performed immunocytochemistry to detect methylated forms of H3K9 and H3K27.

Result: Normal mature neutrophils were immunoreactive to antibodies against methylated histones, which indicated that methylated H3K9 and H3K27 were present in normal mature neutrophils.

Conclusion: This result suggests that epigenetic regulation plays a role in gene silencing during myelopoiesis. We are investigating whether epigenetic mechanisms, such as histone methylation, silence primary granule genes in normal mature granulocytes, which would have implications for the aberrant expression of MPO and PR3 in ANCA patients.

Epidemiology of Primary Systemic Vasculitis and Rheumatoid Vasculitis 1989-2003

R.A. Watts, S.E. Lane, J. Mooney, D.G.I. Scott

Ipswich Hospital, Rheumatology, United Kingdom

Background: Primary systemic vasculitis (PSV) (Wegener's granulomatosis (WG), Churg Strauss Syndrome (CSS), microscopic polyangiitis (MPA)) is rare. The incidence has been reported to have increased after introduction of ANCA testing. The aim of this study was to estimate the incidence of PSV and systemic rheumatoid vasculitis (SRV) in a well defined population.

Methods: We prospectively recorded all cases of PSV and SRV between 1989-2003. The population is 95% Caucasian: 1992 413,500 (200,000 males); 1997 429,000 (207,000 males); 2002 444,500 (214,000 males). The ACR criteria (WG, CSS) and Chapel Hill Consensus Definitions (MPA, WG, CSS) were applied to PSV patients and the Scott and Bacon criteria to SRV patients. Hospital pathology records and discharge coding data were checked to identify missing cases. The annual incidence and 95% confidence intervals (95% CI) were calculated using the Poisson distribution for the observed number of cases.

Results: 128 incident cases (77 male) of PSV were identified in the denominator population during 1989–2003. The median age was 63 yrs (19–90). The overall annual incidence was 19.9/million (95% CI 15.6–24.9). During 1989–1993 the annual incidence was 20.3/million (14.6–27.2), 1994–1998 22.4 (16.5–29.6), 1999–2003 17.1 (12.1–23.5). The incidence was greater in men 24.8/million (19.6–31.0) compared with women 15.3/million (11.4–20.1). A rolling 3 year average revealed no evidence of cyclical peaks or troughs. We identified 66 WG, 37 MPA, and 25 CSS cases. The incidence of WG was 10.2/million (7.9–13.1), MPA 5.8/million (4.0–7.9) and CSS 4.2/million (2.7–6.1). No change in the incidence of WG, MPA or CSS over the 15 yr period, was observed. The peak incidence for all types of vasculitis and PSV as a whole was 65–74 yrs, when the age specific incidence was 58.2/million (42.4–77.8). For comparison we identified 51 (24 male) patients with SRV, during 1989–93 the annual incidence of SRV was 11.6/million (7.4–17.0) and 1999–03 3.6/million (1.6–7.1).

Conclusions: There has been no change in the incidence of PSV since 1989. The study was conducted after the Introduction of ANCA testing in the 1980s, suggesting that the observed increase in incidence during the 1980s was due to increased awareness rather than a change in incidence. In comparison the incidence of SRV fell during the same period.

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Solid Malignancies in the Wegener's Granulomatosis Etanercept Trial

J.H. Stone, J.T. Holbrook, M.A. Marriott, A. Tibbs, L.P. Sejismundo, Y.-I. Min, U. Specks, P.A. Merkel, R. Spiera, J.C. Davis, E.W. St. Clair, J. McCune, S.R. Ytterberg, N.B. Allen

Johns Hopkins University, Rheumatology, Baltimore, USA

Objective: Etanercept is a soluble infusion protein designed to inhibit tumor necrosis factor (TNF). During the Wegener's Granulomatosis Etanercept Trial (WGET), a placebo-controlled trial of etanercept in addition to standard therapy for remission induction and maintenance, an excess of solid malignancies was observed in the etanercept group. In this study we explored further the potential association between TNF and the development of malignancy.

Patients and Methods: A total of 180 patients with active WG were enrolled and followed for a median of 27 months. At enrollment, disease characteristics, treatment history, and specific past medical history items were recorded, as was information about previous WG treatments and risk factors for malignancy.

Results: All 6 solid malignancies observed during the WGET occurred in the etanercept group ($P = 0.01$). These malignancies included 2 cases of mucinous adenocarcinoma of the colon; one each of metastatic cholangiocarcinoma, renal cell carcinoma, and breast carcinoma; and one recurrent liposarcoma. There were no differences between the two groups in gender distribution, disease severity, personal or family history of cancer before WGET enrollment, or tobacco and alcohol use. Based on a comparison of the age- and gender-specific incidence rate for solid malignancies in the SEER database, 1.92 solid malignancies were expected in the etanercept group ($SIR = 3.12\%$; $P = 0.004$). All etanercept-treated patients in this trial who developed solid tumors were also treated with

cyclophosphamide during the trial. Moreover, several had received cyclophosphamide for prolonged periods to treat disease activity prior to enrollment in WGET. Between the two groups, however, there were no differences in the numbers of patients with histories of cyclophosphamide use or the duration or maximum dose of that agent.

Conclusions: Data from the WGET, the first substantial reported experience of the combined use of etanercept and cyclophosphamide, indicate that the combination of TNF inhibition and cyclophosphamide may heighten the risk of cancer beyond that observed with cyclophosphamide alone.

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Predictors of Outcome in ANCA-associated Vasculitis with Severe Renal Involvement: A Prospective Clinico-histopathological Analysis of 100 Patients

R.A.F. de Lind van Wijngaarden, H.A. Hauer, R. Wolterbeek, D.R.W. Jayne, G. Gaskin, N. Rasmussen, L.-H. Noël, F. Ferrario, R. Waldherr, E.C. Hagen, J.A. Bruijn, I.M. Bajema, on behalf of the EUVAS

Leiden University Medical Center, Pathology, Leiden, The Netherlands

Background: This study determined clinical and histological predictors for renal outcome in ANCA-associated vasculitis with severe renal involvement ($\text{crea} > 500 \mu\text{mol/L}$). Because of the severe side-effects of immunosuppressive therapy, there is a need for strategies to modulate treatment regimen, differentiating between disease progressors and non-progressors.

Methods: We performed a prospective analysis of 100 patients, treated either with iv methylprednisolone (IVMeP) or with plasma exchange (PE) as adjunct to standard therapy. Renal biopsies were performed at diagnosis (entry) and GFR0 was established. 39 histological and 10 clinical parameters were determined as candidate predictors for renal outcome. End-points included renal function at 12 months after diagnosis (GFR12) and GFR12 corrected for GFR0 (CORGFR12), dialysis at 12 months and death. A multiple regression analysis was performed.

Results: At entry, 69 patients were on dialysis, while 31 were not. Arteriosclerosis ($r = -0.5$) was predictive of GFR0 in these 31 patients; fibrous crescents ($r = 0.2$) were predictive of dialysis at entry. Of the 69 patients on dialysis, 44% had regained renal function, 32% were on dialysis, and 25% had died after 12 months. Among the 31 patients who were not on dialysis at entry, 65% remained dialysis-independent, 13% ended on dialysis, while 23% had died, after 12 months. Taken the whole group together, normal glomeruli ($r = -0.3$) and treatment limb ($r = -0.3$ {0 = IVMeP, 1 = PE}) predicted for dialysis at 12 months. Normal glomeruli ($r = 0.2$), tubular atrophy ($r = -0.3$), and intra-epithelial infiltrates ($r = -0.3$) were predictors of both GFR12 and CORGFR12. GFR0 ($r = 0.3$) also predicted for GFR12. No parameter predicted for death.

Conclusion: In severe ANCA-associated glomerulonephritis, both chronic and acute lesions were predictors for GFR12, while treatment was predictive of dialysis-dependency after 12 months. A low percentage of normal glomeruli predicted for dialysis at 12 months and a low GFR12.

Sulfamethoxazole Is Effective against Deep Aspergillus Infection in Patients during Immunosuppressive Therapy of ANCA Associated Vasculitis

M. Yoshida, I. Nakabayashi, M. Akashi, T. Watanabe, N. Yoshikawa, S. Hida, K. Ishibashi, N. Ohno

The Department of Renal Unit of Internal Medicine, Hachioji Medical Center of Tokyo

Treatment of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis patients often induces immune suppression. Invasive aspergillosis has significantly increased infrequency among such patients leading to excessive morbidity and mortality. The combination of sulfamethoxazole (SMX) and trimethoprim (TMP) has been used extensively for the treatment and prophylaxis of infections by various microbes. In the present study, we described ANCA-associated vasculitis patients who were treated with SMX-TMP. We also examined anti-aspergillus activity of SMX-TMP. The patient (80-year old, male) was clinically diagnosed as ANCA-associated vasculitis by the titer of MPO-ANCA elevated to 253EU, pulmonary fibrosis and immediately dialysis needed rapidly progressive glomerulonephritis (RPGN). The patient was started to administer only SMX-TMP orally twice a week for prophylaxis to infectious diseases. The patient was once recovered RPGN and discharged hospital, but 6 weeks later he again hospitalized. The patient was unfortunately deceased. By morbid anatomy, fungal ball was observed in lung and *Aspergillus* spp. was detected by cultivation. From the clinical course, suspension of SMX-TMP might strongly relate to the *Aspergillus* infection. To exam the antimicrobial activity of SMX-TMP in vitro, a mixture of SMX and TMP at 5:1 was serially diluted and added to the agar medium. A suspension of *Aspergillus* species was dropped onto the center of the medium. After incubation for one week at 27°C; the diameter of the giant colony was measured. *A. fumigatus*, *A. niger* and *A. oryzae* were used. Colony sizes of all three genus of *Aspergillus* decreased with the increase of SMX-TMP in medium. Thus, sulfonamides may be useful for the prophylaxis of *Aspergillus* infections and control of ANCA-associated vasculitis.

Microscopic Polyangiitis And Disseminated Histoplasmosis

L.F. Flores-Suarez, J.L.L. Zaragoza, J. Baquera

Instituto Nacional de Nutricion, Immunology and Rheumatology, Mexico, D.F., Mexico

Objective: To present the first case of disseminated histoplasmosis in microscopic polyangiitis (MPA). A 63-year-old male with MPA since 1988 presented with arthritis on the left wrist and second right metacarpophalangeal joint in March 2004. In February 2004 he had an acute respiratory infection, believed to be due to a complicated bronchiitis for which he received ciprofloxacin. His MPA was in control although he has non-end stage chronic renal failure and mild

sequelae of peripheral neuropathy. He was in dose reduction of azathioprine (AZA) and steroids according to CYCLOPS trial in which no complications occurred throughout. His latest blood cell count was normal. As he had arthritis and left popliteal swelling he was suspected to have panniculitis, which led to steroid and AZA raise. However, the skin biopsy showed a lobular panniculitis and small vessels neutrophilic vasculitis with intra and extracellular microorganisms in the endothelium. AZA and steroids were stopped. Search for mycobacteria was negative. Serology for *H. capsulatum* (IgM), urinary histoplasma antigen, blood and skin lesion cultures were positive. He was given IV amphotericin but was stopped due to hypotension and fever. He was switched to itraconazole 200 mg bid. On discharge, he continued to improve and gradual decrease of itraconazole followed with total suspension in February 2005. His skin lesions disappeared and full recovery of the arthritis was seen two months after itraconazole treatment. He currently follows a normal life. The MPA is inactive without steroids or immunosuppressives.

Discussion: Disseminated histoplasmosis can reactivate in immunosuppressed patients (v.g. HIV infected). Cases have been seen with the use of anti-tumor necrosis factor for rheumatoid arthritis and occasionally in lupus patients. The mortality of untreated cases can reach 80%. Although initially our patient seemed to have an acute pulmonary infection, he progressed to acute disseminated progressive form although the skin lesions and the arthritis were atypical and no lymphadenopathy or hepatosplenomegaly were seen. During the course of the infection, MPA remained inactive. He has responded well to treatment. In Conclusion, we report the first case of histoplasmosis in the context of MPA. The appearance of skin nodular lesions, arthritis and fever in a patient with MPA obliges inclusion of histoplasmosis as potential cause of the clinical findings.

Severe Life-Threatening Central Nervous System Involvement in Microscopic Polyangiitis: A Case Report

M. Wigger, E. Drückler, J. Muscheites, C. Spang, C. Späthe, D. Haffner

Children's Hospital, Pediatric Nephrology, Rostock, Germany

Background: Central nervous system (CNS) involvement in microscopic polyangiitis (MPA) is a rare symptom and has been reported seldom in the literature. Case report: A 14-year-old girl fell acutely ill with MPA (p-ANCA 1:5200, myeloperoxidase-antibodies (MPO-Ab) > 100 U/ml) that included severe pauci-immune glomerulonephritis resulting in terminal renal insufficiency. The girl was without neurological symptoms. The initial magnetic resonance angiogram (MRA) revealed stenoses of several cerebral vessels, but the magnetic resonance imaging (MRI) was without pathological findings. Immunosuppressive therapy with cyclophosphamide (CP) pulses (500 mg/m²/dose) monthly in combination with corticosteroids in decreasing dosage led to an improvement of MRI-findings. During the 5th month of therapy, generalized seizures occurred, caused by ischemic episodes due to vasculitis (MRI). At that time, p-ANCA presented itself in absence of MPO-Ab like a x-ANCA for the first time. Plasmapheresis and high-dose methylprednisolone pulses improved the clinical

symptoms and MRI-findings. Four weeks later, generalized seizures re-occurred in spite of treatment with corticosteroids (2 mg/kg/d) and CP-pulses. The girl became comatose (Glasgow Scale 5). Both the T2-weighted images, especially the FLAIR-sequence and the native T1-weighted images revealed diffuse cortical and subcortical hyperdensities. After application of gadolinium, images showed a striking patchy meningeal enhancement. The MRA at the same time did not show any stenoses. Altogether, the findings presented a vasogenic edema of the smallest cerebral vessels. After treatment with high-dose methylprednisolone and immunoglobulin (0.5 g/kg/dose), the girl woke up within 12 hours without neurological symptoms. MRI-findings revealed a dramatic improvement with only a few very discreet residues. Under subsequent intensive immunosuppressive treatment with mycophenolate mofetil, cyclosporine A, high-dose methylprednisolone and immunoglobulin, the girl is provided in good condition without any neurological disabilities during 9 months. The patient remains on peritoneal-dialysis treatment.

Conclusion: Severe cerebral episodes of vasculitis during a therapy with CP can be managed by pulses of high-dose methylprednisolone.

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Difficulties in the Long-term Management of Paediatric Patients with Severe Systemic Vasculitis and Fatal Outcome: 2 Case Reports

P. Dolezalova, M. Houstkova, D. Nemcova, Z. Kapounkova, S. Kochanova, J. Hoza

1st Faculty of Medicine, Charles University of Prague, Paediatrics and Adolescent Medicine, Praha, Czech Republic

Objectives: Apart from Henoch-Schönlein purpura and Kawasaki disease primary systemic vasculitides (PSV) are extremely rare in paediatric population. The initial diagnosis is often hampered by hesitation to perform invasive studies in children. Delayed institution of appropriate therapy may be critical for the development of damage which is impossible to reverse. In some cases despite early diagnosis and treatment, persistently active disease evolves into severe chronic disability. The case reports highlight difficulties in 2 areas of PSV management: early diagnosis and long-term complex care.

Methods: Case reports.

Results: Case 1. Seven years old girl presented with cough and chest pain with fevers, malaise, arthralgias and high non-specific inflammatory activity and was treated with multiple antibiotics without effect. Echocardiography performed for a new heart murmur revealed mild tricuspidal insufficiency (TI) considered non-significant. Prednisone therapy led to rapid improvement. About 2 years from the onset while on low-dose prednisone she deteriorated with progressive malaise and dyspnea on exertion. Progression of TI due to severe pulmonary hypertension (PH) was revealed. Multiple hypoperfused areas corresponding with lobar to segmental pulmonary artery occlusions were shown on direct pulmonary angiography. Thrombophilia was excluded. The diagnosis of Takayasu arteritis was made and combined therapy started (corticosteroids,

i.v.cyclophosphamide) together with anticoagulation and continuous O₂ therapy. Nevertheless, PH with congestive heart failure progressed and led to her death 4 years after disease onset. Case 2. Twelve years old girl presented with a year history of generalised livedo following benign streptococcal infection. She also had intermittent arthralgias and fatigue, laboratory tests showed moderate elevation of inflammatory parameters. Skin biopsy revealed necrotising vasculitis of deep dermal vessels. Diagnosis of cutaneous polyarteritis nodosa (PAN) was made. She was treated with oral Penicillin and low-dose prednisone, which was weaned over 8 months, low-dose aspirin and nifedipine. Her general complaints settled but livedo persisted. When Penicillin was stopped 4 years from the onset she flared with anorexia and abdominal pain with progressive weight loss and increase of non-specific inflammatory parameters.

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Establishment of the Evidence of Beneficial Effect of Intravenous Immunoglobulin (IVIg) Therapy on MPO-ANCA Related Polyangiitis Combining Rapidly Progressive Glomerulonephritis (RPGN) in Japan

E. Muso, T. Ito-Ihara, T. Ono, E. Imai, K. Yamagata, A. Akamatsu, K. Suzuki

Kitano Hospital The Tazuke Kofukai Medical Research Institute, Osaka, Japan

ANCA-related RPGN often necessitated aggressive immunosuppressive treatment which sometimes brought about severe side effects such as fatal infections, especially in relatively aged population who has high incidence of this disease. To avoid these fatal side effects, IVIg therapy has been utilized mainly in EU and USA, however, the trial and survey of this therapy in Japan where the epidemiologically distinguished from EU with characteristically high incidence of MPO-ANCA related disease has not been widely accepted. To evaluate the effectiveness of IVIg in MPO-ANCA related RPGN, 1) survey of the outcome of 32 patients (male 19, female 13, average age of 68 years old), treated by IVIg (400 mg/kg/day) for five consecutive days prior to or along with conventional immunosuppressive therapy and 2) planning for the randomized placebo-controlled multicenter double blind study were firstly performed in Japan. In survey of the 32 patients, 20 patients who were treated with IVIg independently before the start of conventional therapy showed significant decrease of CRP from 8.61 ± 5.77 to 5.47 ± 4.50 mg/dl ($P < 0.001$) with improvement of elevated serum creatinine (Cre) in 12 out of 19 patients (63%). Immunosuppressive therapy after or along with IVIg were started using relatively low dose of prednisolone (PSL). For 22 out of 32 patients, administration of PSL less than 0.5 mg/kg/day was sufficient to induce the remission. As for the outcome at 6 months period, after excluding the 4 cases who have had been induced the hemodialysis before IVIg, high renal survival rate (92.3%) was obtained. Although 2 patients died (survival rate: 93.7%) due to cerebral bleeding before 6 months, no fatal systemic infection was reported. Following these favorable Results early phase II study to establish the evidence of the beneficial effect of IVIg is now on going.

The Usefulness of Formalin-fixed Neutrophils in Antineutrophil Cytoplasmic Antibody (ANCA) Testing

W. Pollock

University of Melbourne, Department of Medicine,
Melbourne, Australia

Aims: To evaluate the usefulness of formalin-fixed neutrophil slides for indirect immunofluorescence (IIF) screening for antineutrophil cytoplasmic antibody (ANCA) in different ANCA-associated diseases.

Methods: IIF was performed using ethanol and formalin fixed slides (INOVA Diagnostics Inc, CA, USA), and sera from patients with biopsy-proven Wegener's granulomatosis (WG) (n = 10) or microscopic polyangiitis (MPA) that was 'active' (within 3 months of presentation or clinical relapse) (n = 25) or 'treated' (at least 3 months after initiation of treatment) (n = 24). Sera were also tested from patients with ulcerative colitis (n = 15), Crohn's disease (n = 7) or systemic lupus erythematosus (SLE, n = 19). Controls were hospital patients with suspected vasculitis or normal blood donors who were IIF-positive on ethanol-fixed slides (n = 22). All sera were tested for myeloperoxidase (MPO) ANCA and proteinase 3 (PR3) ANCA by various commercial and in-house assays and were deemed positive by consensus.

Results: Twenty of the 21 patients (95%) with active MPA and perinuclear fluorescence specific for MPO demonstrated cytoplasmic fluorescence on formalin-fixed slides. However 6 of the 20 patients (30%) with treated MPA and perinuclear fluorescence became negative by IIF on formalin-fixed slides as did all (12/12) of the patients with ulcerative colitis and 9 of the 16 patients (56%) with SLE.

Conclusion: Formalin-fixed cells demonstrated negative fluorescence in all patients with ulcerative colitis and cytoplasmic fluorescence in most patients with active MPA all of who had perinuclear fluorescence on ethanol-fixed cells, but was less helpful in patients with treated MPA and in SLE. Confirmation of positive IIF by antigen-specific ELISA is more useful than retesting on formalin-fixed cells.

Serial ANCA Determinations for Monitoring Disease Activity in Patients with ANCA-Associated Vasculitis: Systematic Review

R. Birck, W.H. Schmitt, A.I. Kalsch, F.J. van der Woude

University Hospital Mannheim, Fifth Department of
Medicine, Mannheim, Germany

Background: Studies investigating the utility of serial ANCA monitoring for assessing relapsing disease activity in patients with ANCA-associated vasculitis (AASV) have yielded controversial results over the last 15 years. To assess the diagnostic value of serial ANCA testing in the follow up of AASV patients we conducted a systematic review of the available literature.

Methods: Studies were identified by a comprehensive search of the PubMed, BIOSIS+/RRM, and EMBR databases. The

methodological quality of all eligible studies was assessed according to established criteria for diagnostic studies. Contingency tables were built when possible for calculation of individual and pooled measures of test accuracy. Sensitivity analysis according to a priori defined variations in study methodology was conducted to investigate potential sources of heterogeneity.

Results: Twenty studies met our inclusion criteria including a total of 917 patients. Qualitative analysis revealed methodologic flaws mainly with respect to the internal validity in the majority of studies. Major problems were selection and review bias. Besides considerable methodological heterogeneity, chi square tests indicated substantial statistical heterogeneity as well for all measures of diagnostic accuracy making meta-analytic pooling not useful. Sensitivity analysis, which was only possible in a subset of studies (n = 6), could not identify sources of heterogeneity.

Conclusion: The presence of design-inherent methodologic flaws makes the results of the majority of included studies susceptible to bias. Meta-analytic pooling was not useful due to substantial methodological and statistical heterogeneity. In our opinion the available literature does not allow to draw firm and reliable conclusions concerning the usefulness of serial ANCA determinations for monitoring disease activity in AASV patients.

Evaluation of Commercial Direct and Capture Immunoassay Kits for Detection of PR3-ANCA

J.U. Holle, B. Hellmich, W.L. Gross, E. Csernok

University Clinic of Schleswig-Holstein,
Dept. of Rheumatology, Lübeck, Germany

Objective: To compare the performance characteristics of three capture- and 12 direct-ELISA commercial kits for quantification of PR3-ANCA in well characterized patients with ANCA-associated vasculitides (AAV).

Material and Methods: Serum samples were derived from patients with histological and clinical diagnosis of Wegener's granulomatosis (WG, n = 15), microscopic polyangiitis (n = 15), other primary or secondary vasculitides (n = 10), disease controls (n = 30) and healthy controls (n = 10). Each of them was tested for the presence of ANCA by indirect immunofluorescence technique (IFT), direct and capture ELISA. Diagnostic performance of the tests was estimated by receiver operating characteristic (ROC) curve analysis and sensitivity and specificity in detection of ANCA/PR3-ANCA were calculated for the respective methods.

Results: Applying the cut-off provided by the manufacturers, there were great variations among the sensitivities of the commercial direct PR3-ANCA kits (ranging from 13.3 to 66.7%) and capture ELISAs (ranging from 53.3 to 60%) for a diagnosis of WG, whereas specificities were relatively constant (96.0 to 100% and 98.5 to 100%, respectively). Performance measured by AUC values ranged from 0.724 to 0.900 among direct ELISAs and from 0.789 to 0.877 among capture ELISAs. There were no significant differences comparing AUC values of direct ELISAs to capture ELISAs. The performance of most ELISAs could be increased by lowering cut-off levels, thereby increasing sensitivity and AUC and lowering specificity.

Conclusion: The results indicate that the sensitivities for direct PR3-ANCA ELISA differ among the commercial kits tested, whereas the capture ELISA results showed a significant correlation between all assays. Diagnostic performance of capture ELISAs for detection of PR3-ANCA in AAV is similar to the direct ELISA and IFT.

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Standardisation of Testing for Antineutrophil Cytoplasm Antibody (ANCA)

W. Pollock, Gribbles Pathology, Clayton, J. Savige

University of Melbourne, The Northern Hospital, Epping, AUSTRALIA and the Australasian ANCA Study group

Aims: The International Consensus Statement on Testing and Reporting Antineutrophil Cytoplasmic Antibodies (ANCA) and its Addendum indicate the minimal and optimal requirements for ANCA testing in patients with suspected vasculitis (Savige et al., 1999, 2003). We have surveyed all testing laboratories in Australasia to determine whether they have adopted these guidelines.

Methods: The survey was distributed to the 43 laboratories enrolled in the Quality Assurance Programme for ANCA testing of the Royal College of Pathologists of Australasia.

Results: Twenty-three laboratories (53%) responsible for 14,500 ANCA assays during the previous 3 months responded. All 23 screened for ANCA by indirect immunofluorescence (IIF), and 15 (15/17, 88%) confirmed all IIF-positive sera in ELISAs for both antiproteinase and antimyeloperoxidase antibodies. A further 4 laboratories (17%) performed IIF plus both ELISAs on all sera. These 23 laboratories used 5 different IIF substrates and 11 different ELISAs (including inhouse). Almost all laboratories (20/23, 87%) examined ethanol-fixed neutrophils and the remaining 3 (13%) used formalin-fixed cells in addition to differentiate between IIF patterns. Nine (39%) laboratories titred their IIF. Twenty-two laboratories (96%) reported C-ANCA, P-ANCA and interfering ANA, and 16 (73%) reported atypical and 14 (64%) atypical C ANCA patterns. Eleven (11/21, 55%) reported their ELISA results using only quantitative units. Only 7 laboratories (7/18, 40%) used the Consensus comments and 3 (17%) did not make comments at all. Eight of the 12 laboratories (8/12, 66%) that reported IIF before the ELISA results indicated in their comments that neutrophil fluorescence alone was not specific for the diagnosis of Wegener's granulomatosis or microscopic polyangiitis.

Conclusions: Almost all Australasian diagnostic testing laboratories screen for ANCA by IIF and confirm positive results using ELISAs for both antiproteinase and antimyeloperoxidase antibodies. Many different IIF and ELISAs are used to test for ANCA. Laboratories vary in how they report their ELISA results and use of interpretive comments which complicates the clinician's task in following patients tested in different laboratories.

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Evaluation of Antineutrophil Cytoplasmic Antibody Assays for Proteinase 3 and Myeloperoxidase in Vasculitis and Inflammatory Bowel Disease

J. Savige, M. Trevisin, W. Pollock, W. Dimech, J. Melny, B. Paspaliaris, D. Gillis, R. Wong

Diagnostic Immunology Laboratory, Austin Hospital, Australia and the Australasian ANCA Study Group

Aim: To evaluate 12 commercial and in-house assays (including one capture ELISA) for the detection of antineutrophil cytoplasmic antibodies (ANCA) directed against proteinase 3 (PR3) and myeloperoxidase (MPO) in patients with Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and in inflammatory bowel disease (IBD).

Patients and Methods: All assays were evaluated using the Australian National Committee for Clinical Laboratory Standards protocol. Patients had biopsy-proven WG or MPA that was 'active' (at presentation) or 'treated' (at least 3 months after treatment started), or IBD that was ANCA-positive by IIF (n = 23). Controls were hospital patients with suspected vasculitis (n = 35), with known systemic lupus erythematosus (n = 20), or normal blood bank donors (n = 33).

Results: The median coefficients of variation for the PR3- and MPO-ANCA ELISAs were <10% within and <20% between assays. All assays demonstrated good linearity and the median binding values for the 2 standards were 128 Units (range 27–1386) for the PR3-ANCA and 60 Units (range 10–179) for the MPO-ANCA assays. The median sensitivity for the combination of PR3-ANCA and MPO-ANCA ELISAs for active vasculitis was 94% (range 91–96%). The median specificity for the PR3-ANCA assays was 94% (range 88–96%) and for MPO-ANCA, 97% (range 86–99%). ROC analysis using the area under the curve ranged from 0.929 to 0.979 for PR3-ANCA ELISAs and from 0.846 to 0.962 for MPO-ANCA ELISAs. All assays detected ANCA in at least one patient with IBD. One detected PR3-ANCA in 10/23 (43%) patients and another detected MPO-ANCA in 7/23 (30%) patients.

Conclusions: The PR3- and MPO-ANCA ELISAs were highly sensitive for active WG and MPA but less sensitive for treated disease. The same assays varied in their ability to detect ANCA in IBD.

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Evaluation of a New Fluorescence Immunoassay for Detection of Antibodies against Proteinase-3 and Myeloperoxidase

C. Cerveira, O. Figueiras, P. Carneiro, E. Santos

Serviço Imunologia, Hospital Geral Santo Antonio, Porto, Portugal

Objective: To compare the results of a new fluorescence immunoassay (EliATM) for antibodies to proteinase-3 (PR3) and myeloperoxidase (MPO) with those obtained with a reference commercially available ELISA; to evaluate the diagnostic performance of both methods against the standardized indirect immunofluorescence test (IIF) for antineutrophil cytoplasmic antibodies (ANCA) in patients with clinically defined ANCA associated systemic vasculitis (AAV). Patients: 162 sera samples were analyzed: 84 patients with AAV: 27 Wegener's Granulomatosis (WG), 7 Microscopic Polyangiitis, 41 Rapidly Progressive Glomerulonephritis and 9 other AAV; the disease control group (n = 78) consisted of 67 patients with chronic inflammatory diseases and 11 with acute infectious diseases; as healthy controls a group of 15 blood donors were tested and were negative in any of the tests.

Results: The clinical significance of the results for all the patients showed a slightly better sensitivity (53.6%) and specificity (88.5%) of the combination of EliATM PR3/MPO for AAV compared to the combination of ELISA PR3/MPO (44.1% sensitivity, 83.3% specificity). As expected, ANCA IIF test had a better sensitivity (66.7%) and a lower specificity (50.0%). The tests performed on patients with WG revealed, as most of them were in remission, a rather low sensitivity for the combination of c-ANCA/PR3 (48.2% for EliATM, 33.3% for ELISA) and even 70.4% for c-ANCA IIF alone. However, specificity of those combinations for WG was respectively 87.7% and 91.2% in the group of AAV patients, and 92.6% and 94.9% overall.

Conclusions: The automated fluorescence immunoassay EliATM for detection of antibodies to PR3 and MPO in patients with AAV demonstrated a good sensitivity and a very good specificity, comparable with other commercially available assays.

Sensitive Detection of Myeloperoxidase Expression on Neutrophil of Patients with Myeloperoxidase-antineutrophil Cytoplasmic Antibody-associated Glomerulonephritis

T. Ito-Ihara, K. Uno, A. Hoshino, K. Yamamoto, T. Komiya, A. Ishida-Okawara, A. Fukatsu, T. Kita, K. Suzuki, E. Muso

Graduate School of Medicine, Kyoto University, Nephrology and Cardiovascular Medicine, Kyoto, Japan

Ultrafine nanocrystals are expected to be used widely in biotechnology and medical applications. Quantum dots (QDs) are novel inorganic fluorophores that consist of CdSe/ZnS-core/shell semiconductor nanocrystals. QDs show high luminance which is resistant to photobleaching, with a range of excitation wavelengths from ultraviolet to

red depending on the size of the particles. In this study, to establish and evaluate the facile and sensitive method to detect activated neutrophils in patients of MPO-ANCA-associated glomerulonephritis (GN), we tried to visualize surface expression of MPO on neutrophils of patients or of healthy controls, using QD conjugated antibody against MPO.

Methods: Neutrophils (2×10^6 cells/ml) obtained from five patients with MPO-ANCA-associated GN and from five healthy controls, were adhered on the coverslips preincubated at 37° C. The adherent neutrophils were stimulated with FMLP or TNF-alpha for 10 minutes at 37° C and fixed with 4% paraformaldehyde after washing with chilled PBS. After blocked with 1% bovine serum albumin for one hour at room temperature cells were stained with rabbit anti-mouse MPO QD-immunoconjugates.

Results: In healthy controls, surface MPO expression was not apparent in unstimulated neutrophil, however emerged expression was visualized with TNF-alpha or FMLP stimulation. On the other hand, in patients, marked staining of MPO was observed on the surface of even unstimulated neutrophils.

Conclusion: The sensitive detection of surface-expressed MPOs on the neutrophil may contribute to the evaluation of disease activity in MPO-ANCA related GN.

Contribution of Myeloperoxidase (MPO) on Surface of Neutrophils to Glomerulonephritis Using Nanocrystal Quantum Dots-labeled MPO Antibody

A. Hoshino, T. Nagao, K. Murayama, A. Ishida-Okawara, T. Ito-Ihara, K. Uno, E. Muso, N. Nagi-Miura, N. Ohno, S. Naoe, K. Tokunaka, M. Yasuhara, K. Yamamoto, K. Suzuki

National Institute of Infectious Diseases, Dept. Bioactive Molecules, Tokyo, Japan

We examined the role of myeloperoxidase (MPO) in the pathogenesis of glomerulonephritis associated with MPO-specific anti-neutrophil cytoplasmic autoantibody (MPO-ANCA) in a murine model using nanocrystal quantum dots (QDs). We demonstrated that QD-conjugated anti-recombinant mouse MPO (rmMPO) Ab enable to visualize the expression of MPO on the surface of neutrophils after stimulated with FMLP or some proinflammatory cytokines. Notably, expressions of surface MPO on non-stimulated neutrophils were enhanced in both the patient with MPO-ANCA-related glomerulonephritis and SCG/Kj mice showing spontaneous crescentic glomerulonephritis. Moreover, the initiation of inflammatory vascular lesions in C57BL/6 mice was induced by intraperitoneal injection of *Candida albicans* water-soluble polysaccharides (CAWS) in the presence or absence of anti-rmMPO Abs. Simultaneous administration of anti-rmMPO Abs with CAWS significantly promoted the severe collapse of glomeruli, and QD-labeled anti-rmMPO Abs were accumulated on the neutrophils infiltrated into collapsed glomeruli, but not control IgG and CAWS. Furthermore, blood flow in kidney surface vessel was significantly made slow-down in SCG/Kj mice and CAWS-treated mice, suggesting that CAWS and anti-MPO antibody may cause damage in blood vessel continuing the formation and development of glomerulonephritis. These results indicate that

surface-expressed MPO on the activated neutrophils with anti-MPO Abs may coordinately play essential roles in the initial steps for the development of glomerulonephritis.

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Anti-neutrophil Cytoplasmic Antibodies in Severe Infection – A Neglected Association

V. Schwenger, C. Morath, J. Sis, M. Haensch, M. Zeier, R. Waldherr, K. Andrassy

University of Heidelberg, Medicine 1, Heidelberg, Germany

Background: C-ANCA with target antigen proteinase 3 (PR3-ANCA) and P-ANCA with target antigen myeloperoxidase (MPO-ANCA) are highly sensitive and specific markers for systemic small vessel vasculitides with renal involvement (M. Wegener and microscopic polyangiitis). Although ANCAs have been considered to be highly specific for vasculitides, positive ANCAs have also been observed in patients with severe systemic infections.

Objective: Recently, we observed a female veterinary medical student who was admitted with pneumonia, rapidly progressive glomerulonephritis (RPGN) (S-creatinine 3 mg/dl) and highly elevated ANCA (P-ANCA 1:1260, nv: <1:20, MPO 33, nv <2), which turned out to be acute Q-fever infection (coxiella IgG 504 U/L; IgM +++). Renal histology revealed crescentic and necrotic immune complex nephritis (postinfectious glomerulonephritis). Under long-term treatment with doxycycline and levofloxacin, coxiella titers declined slowly from 504 U/L to 90 U/L; within 6 months renal function improved (S-creatinine) from 3 mg/dl to 1.3 mg/dl and proteinuria decreased from 3.1 g/day to 1.2 g/day. Although crescentic RPGN is usually treated by immunosuppression, we avoided this therapy in our patient because of possible harmful effects on the course of Q-fever infection. Whereas Q-fever infection was cured with this regimen, renal function declined progressively, becoming strongly impaired in the follow-up. A second renal biopsy revealed chronic sclerosing glomerulopathy with advanced tubulo-interstitial fibrosis. Discussion: ANCA with the classical target antigens PR3 or MPO in infectious diseases were shown in patients with endocarditis, HIV, amoebiasis, leprosy, tuberculosis, syphilis and hepatitis C. Here we add Q fever as another granulomatous infectious disease. ANCA with other target antigens, e.g. elastase and BPI (bacterial permeability inhibitor), could be demonstrated in other bacterial infections (e.g. osteomyelitis, cystic fibrosis).

Conclusion: 1. ANCA are not only markers of small vessel vasculitides, but can also be (false?) positive in patients with systemic infection. 2. Determination of target antigens for ANCA is indispensable. 3. Interpretation and therapeutic decisions concerning ANCA associated diseases should only be performed within the clinical context.

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Association of Clinical Autoimmune Manifestations with Autoantibodies in Patients with Hepatitis C Virus (Hcv) Infection under Treatment with Interferon-alpha

W.H. Schmitt, C. Antoni, P. Schnülle, K.-P. Becker, B.A. Yard, M. Singer, F.J. van der Woude

University Clinic Mannheim, 5 Medizin, Mannheim, Germany

Introduction: HCV infection has been found to be clinically associated with autoimmune phenomena, and various autoantibodies have been described in this condition. Furthermore, treatment with interferon α (IFN α) seems to induce autoantibody formation. However, the association of clinical autoimmune phenomena with these antibodies is still unclear.

Patients and Methods: 101 consecutive patients with chronic hepatitis C, who had been treated with IFN α for 14.3 ± 12.7 weeks, were prospectively screened for clinical autoimmune phenomena. Sera ($n = 208$) were assayed for various autoantibodies including anti-nuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA) and anti-thyroid antibodies.

Results: Clinical autoimmune phenomena, observed in 27 cases, were rather mild in nature (hairloss, sicca syndrome, arthralgias, myalgias, thyroid dysfunction), did not affect vital organs beside the thyroid gland, and were associated with the duration of treatment ($P = 0.03$). Female sex (odds ratio (OR) = 12.8; 95% CI, 2.9–23.3), ANA of a titer $\geq 1:640$ (OR 6.9; CI, 2.1–23.1), ANCA (OR 3.5; CI, 1.2–10.5) and anti-thyroid antibodies (OR 6.1; CI, 1.6–23.0) were significantly associated with clinical autoimmune phenomena and were confirmed by multivariate analysis as independent predictors. No specificities of ANA and ANCA and no significant time relationship between the duration of IFN α exposure and the occurrence of autoantibodies could be detected, although there was a trend for increasing ANA titers under treatment.

Conclusion: Beside thyroid dysfunction, clinical autoimmune phenomena in patients with HCV infection under therapy with IFN α were mostly mild. Their occurrence was significantly associated with the presence of ANA (titer $\geq 1:640$), ANCA and anti-thyroid antibodies and the duration of treatment with IFN α .

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Detection of Renal ANCA-associated Vasculitis by Urinary Proteomic Analysis

M. Haubitz¹, S. Wittke², A. Woywodt¹, H.D. Ruppert, H. Haller¹, H. Mischak²

¹Dept. of Nephrology, Medical School ²Mosaiques Diagnostics, Dept. of Nephrology, Ludwig Maximilians University, Munich, Germany.

Objective: Renal manifestation of ANCA-associated vasculitis is characterized by a necrotizing pauci-immune glomerulonephritis. Up to now diagnosis could only be achieved by renal biopsy. Recently

a novel high throughput method, capillary electrophoresis on-line coupled to a mass spectrometer (CE-MS), has been developed allowing fast evaluation of up to 2000 polypeptides in one urine sample. This method, able to give typical urinary-patterns in patients with different glomerular diseases, was applied to patients with an ANCA-associated renal vasculitis to establish a pattern for diagnosis and monitoring of renal disease activity. Method.: 25 patients with ANCA-associated vasculitis, 11 with active renal disease and 14 in remission were measured. In addition, serial measurements were done in 8 patients before, 1, 3 and 6 months after initiation of immunosuppressive treatment with prednisolone and cyclophosphamide.

Results: Using support vector machines, a specific polypeptide pattern was identified by CE-MS that segregated patients with ANCA-associated renal vasculitis from healthy controls and patients with other glomerular disease like IgA nephropathy, membranous nephropathy, minimal-change disease, focal-segmental glomerulosclerosis and diabetic nephropathy. Moreover, patients with active renal disease could be distinguished from patients in remission. After initiation of treatment urinary pattern changed during 6 months from active vasculitis to vasculitis in remission. Prospective studies, regarding the sensitivity and specificity of the method to detect active renal ANCA-associated vasculitis are currently underway.

Conclusion: Detection of active ANCA-associated renal vasculitis by CE-MS is a promising non-invasive tool for the surveillance of patients with ANCA-associated vasculitis. Identification of specific polypeptides might also be useful regarding the pathogenesis of the disease.

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Cloning and Expression of Recombinant c-myc Tagged Human Neutrophil Elastase and its Use as Target Antigen in a Novel Capture ELISA for Detection of Elastase Antineutrophil Cytoplasmic Antibodies

T. Peikert, M.A. Viss, A.M. Hummel, M. Trimarchi, G. Gregorini, U. Specks

Mayo Clinic Pulmonary and Critical Care Medicine, Rochester, USA

Objectives: Cocaine-induced midline destructive lesions (CIMDL) are rare complications of cocaine abuse, characterized by extensive destruction of osteocartilaginous structures of nose, sinuses and palate. Antineutrophil cytoplasmic antibodies reacting with human neutrophil elastase (HNE-ANCA) emerged as a marker for CIMDL. Because of variable sensitivity multimodality testing to detect HNE-ANCA remains advisable. To facilitate and optimize assay methodology for HNE-ANCA detection, we expressed recombinant, enzymatically inactive, c-myc tagged human elastase (rHNE-cmyc) and used it as target antigen in a novel capture ELISA.

Methods: Full length cDNA of wild type HNE, modified to contain the active site mutation S195A and deletion of the N-terminal activation dipeptide, was sub-cloned into the expression vector pcDNA4/myc-His and stably transfected into the human embryonal kidney cell line 293.

Results: Expression of rHNE-cmyc was verified by ELISA. rHNE-cmyc was captured with a monoclonal anti-cmyc antibody and detected with a polyclonal sheep anti-HNE antibody. Furthermore, the protein was detected by Western blot using a rabbit polyclonal anti-HNE and a monoclonal anti-cmyc antibody. rHNE-cmyc migrates as a 35 kDa double band consistent with the predicted size of 301 amino acids and Asn-linked glycosylation. The capture ELISA using rHNE-cmyc as target antigen generated a positive signal in serum samples from HNE-ANCA positive patients with CIMDL.

Conclusion: rHNE-cmyc protein secreted into 293 cell supernatants has the conformation of the mature enzyme, but lacks enzymatic activity. Therefore, it does not need to be inactivated prior to use in solid phase assays for HNE-ANCA detection. Furthermore, anchoring via the carboxy-terminal tag optimizes accessibility of potential HNE-ANCA epitopes.

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Long Term Prognosis in Renal Vasculitis with Anti-MPO Antibodies

J.P. Devaux, C. Pagnoux, X. Kyndt, P. Bataille, V. Lemaitre, P. Vanhille, L. Guillevin

Valenciennes, Boulogne, Paris, France

The aim of the study was to determine the renal prognosis and the pattern of relapses in renal vasculitis with anti-MPO antibodies.

Patients and methods: Forty-one patients (mean age 61 years), with histologically proven renal involvement were retrospectively included. The induction treatment combined corticosteroids and cyclophosphamide, and was followed by a maintenance therapy. The mean duration of follow-up was of 58 months. The activity of the vasculitis (BVAS score) was assessed monthly during one year, then four times a year. The glomerular filtration rate (GFR) was calculated (Cockcroft and Gault formula) at the time of the diagnosis (T0) and at each visit during the follow-up. ANCA were determined using the standardised immunofluorescence assay, and ELISA.

Results: Among the 41 patients (median GFR at T0 = 33 ml/mn), a renal remission was observed in 37 patients after a median of 7.2 months. Twenty patients presented one or more relapse (mean 2.45 relapses), which occurred after a median period of 27 months. The evolution to severe or moderate renal insufficiency was affected by the number of organs involved and the BVAS at T0. A poor renal prognosis was also associated with the creatinine clearance ($p = 0.056$), proteinuria ($p < 0.05$), and hypertension ($p = 0.056$) at T0, hypertension ($p = 0.001$) at 1 month, and the duration of ANCA positivity. Persistent anti-MPO antibodies were the unique parameter predicting relapses ($p = 0.015$).

Conclusions: In anti-MPO associated renal vasculitis, the renal parameters, the extent and the severity of the vasculitis process at T0 were associated with a poor renal outcome. This latter was also influenced by the duration of ANCA positivity, which represented the unique parameter associated with relapses. Our results suggested that persistent anti-MPO Ab should be closely monitored after withdrawal of immunosuppressive drugs. The treatment protocols should take account of ANCA positivity for this group of patients.

Urinary Matrix Metalloproteinases Reflect Renal damage in Anti-Neutrophil Cytoplasm Autoantibody-associated Vasculitis

J.S.F. Sanders, M.G. Huijtema, R. Hanemaaijer, H. van Goor, C.G.M. Kallenberg, C.A. Stegeman

University Medical Center Groningen, Clinical Immunology, Groningen, The Netherlands

Objective: Renal expression of MMP-2, -9, and Tissue Inhibitor of MMP-1 (TIMP-1) correlates with disease-activity in ANCA-associated vasculitis. We studied whether urinary and plasma MMP 2, -9, and TIMP-1 reflect renal expression and disease-activity in ANCA-associated vasculitis.

Methods: Urine and plasma of patients with ANCA associated vasculitis who underwent a renal biopsy was collected (n = 32). Urinary activity of MMP-2, and -9 was measured by activity assays. Urinary and plasma levels of MMP-2, MMP-9 and TIMP-1 were measured by ELISA. Healthy controls provided plasma and urine for comparison (n = 31). In patients urinary and plasma levels were related with clinical and histological disease activity, and renal expression of MMP-2 and MMP-9. Immunohistological MMP expression was compared with expression in normal renal tissue (n = 8).

Results: Urinary MMP-2, MMP-9 activity and urinary and plasma TIMP-1 levels were significantly higher in patients than in controls. In glomeruli of patients more MMP-2-, but less MMP-9 expressing cells were present than in control sections. Glomerular MMP expression reflected glomerular inflammation. Urinary activity of MMP-2, and MMP-9 did not correlate with renal expression or plasma levels. Urinary MMP activity correlated negatively with glomerular inflammation, but positively with fibrous crescents. Urinary MMP-2 and TIMP-1 correlated with tubulointerstitial damage and clearance at biopsy.

Conclusion: Urinary MMP-2, MMP-9, and TIMP-1 are elevated in ANCA-associated renal vasculitis but do not reflect renal MMP expression and inflammation. However, urinary MMP-2 activity and TIMP-1 levels reflect tubulo-interstitial damage, and correlate with creatinine clearance at biopsy.

Urinary MCP-1 as a Potential Prognostic Marker in ANCA-Associated Small Vessel Vasculitis

S. Ohlsson, O. Torfvitt, O. Bakoush, J. Tencer, M. Segelmark

Kidney Research Laboratory, Nephrology, Lund University, Sweden

Aims: The ANCA-associated vasculitides (AASV) are diseases of relapsing-remitting inflammation. MCP-1 have been shown to be locally upregulated in glomerulonephritis and recent studies have

pointed out MCP-1 as a promising marker of renal inflammation. Here we measure urinary cytokine levels in different phases of disease, exploring the possible prognostic value of MCP-1, together with IL-6, IL-8 and IgM. Major findings: The urinary MCP-1 levels were significantly higher in patients in stable phase of the disease (n = 82), compared with healthy controls (n = 14) (1.7, undet-19.1 vs 1.0, undet-2.9 pg/mmol creatinine, $p < 0.05$). Patients in stable phase, with subsequent adverse events had significantly higher MCP-1 values than patients who did not (7.2, 0.6–14.5 vs 1.5, undet-19.1 pg/mmol creatinine, $p < 0.001$). MCP-1 and IgM both tended to be higher in patients relapsing within three months, an observation, however, not reaching statistical significance. Urinary levels of IL-6 and IL-8 did not seem to have any value as markers of disease activity or outcome.

Principal conclusions: Patients with AASV have raised cytokine levels in the urine compared to healthy controls, even during remission. Raised MCP-1 and possibly IgM levels seem associated with poor prognosis and relapse tendency. Further studies are needed in order to confirm our results.

Anti-oxLDL Antibodies in MPO-ANCA Vasculitis Patients Preferentially Recognize Epitopes on Hypochlorite-modified LDL

J. Damoiseaux, J.W.C. Tervaert, M. Slot, R. Theunissen, P. van Paassen

Academic Hospital Maastricht, Clinical and Experimental Immunology, Maastricht, The Netherlands

Many patients surviving vasculitis are prone to accelerated atherosclerosis and often have enhanced levels of antibodies to oxidized LDL (oxLDL). To measure anti-oxLDL antibodies, oxidation of LDL is normally achieved with copper (Cu) or malondialdehyde (MDA). Alternatively, LDL may be oxidized with myeloperoxidase (MPO) or its product hypochlorite. Because in active vasculitis MPO is released into the plasma, the latter pathway might play a dominant role in ANCA-associated vasculitis (AAV), and might be different between AAV patients with MPO- or PR3-ANCA. In the present study we determined the best substrate, i.e. MDA-LDL, Cu-LDL and hypochlorite-LDL, for measuring anti-oxLDL antibodies by ELISA. Results were compared between AAV patients (n = 89) and healthy controls (HC; n = 38), and between patients with MPO-ANCA (n = 43) and PR3-ANCA (n = 46). Optimal cut-off points were determined by ROC-curve analysis. Results revealed that anti-oxLDL antibodies are enhanced in AAV patients due to increased levels in patients with MPO-ANCA as compared to patients with PR3-ANCA. Hypochlorite-LDL was by far the best substrate to establish these differences. These results probably reflect enhanced MPO-mediated LDL oxidation in patients with MPO-ANCA. The presence of circulating anti-MPO ANCA, which can prevent inactivation of MPO activity by ceruloplasmin, is suggested to account for this effect.

Circulating Endothelial Cells in Relapse and Granulomatous Upper Airway Disease in ANCA-associated Small-vessel Vasculitis

A. Woywodt, C. Goldberg, T. Kirsch, K. de Groot, H. Regelsberger, U. Erdbruegger, H. Haller, M. Haubitz

Hannover Med School, Nephrology, Hannover, Germany

Objective: To evaluate numbers of circulating endothelial cells in patients with relapse and limited granulomatous upper airway disease in small-vessel vasculitis associated with anti-neutrophil cytoplasmic antibodies (ANCA).

Methods: The study sample included 16 patients with relapse of ANCA-associated vasculitis, six patients with newly diagnosed acute vasculitic disease and 12 patients with limited granulomatous upper airway disease due to Wegener's granulomatosis. 20 patients in remission were also studied, as were 20 healthy controls. Enumeration of circulating endothelial cells was performed with anti-CD 146-driven immunomagnetic isolation and subsequent staining with Ulex Europaeus lectin I (UEA-1). We also did monthly follow-up measurements in patients with relapse.

Results: Patients with relapse of vasculitis had grossly elevated numbers of circulating endothelial cells (12–800 cells/ml, median 134 cells/ml) as did patients with newly diagnosed systemic vasculitis (8–216 cells/ml, median 54 cells/ml). Patients with limited granulomatous upper airway disease due to Wegener's granulomatosis had slightly elevated cell numbers (4–44 cells/ml, median 16 cells/ml, $p = 0.17$ when compared to patients in remission). Patients in remission had slightly elevated cell numbers (4–36 cells/ml, median 16 cells/ml). Healthy controls had very low cell numbers (0–16 cells/ml, median 4 cells/ml). Cell numbers in patients with relapse and new-onset vasculitis declined with successful immunosuppressive treatment.

Conclusion: Elevated numbers of circulating endothelial cells indicate relapse in ANCA-associated small-vessel vasculitis and discriminate vasculitic disease from granulomatous inflammation. These findings add further proof to the idea of circulating endothelial cells as a marker of ANCA-associated small-vessel vasculitis.

High Urine IgM Excretion at Diagnosis Predicts Outcome in ANCA-associated Renal Vasculitis

M. Segelmark, O. Bakoush, O. Torffvit, S. Ohlsson, J. Tencer

Lund University, Nephrology, Lund, Sweden

Objective: Renal function at diagnosis is a strong predictor not only for renal survival but also for patient survival in patients with ANCA-associated small vessel vasculitis (ASVV). However, apart from renal function there are no other established risk factors for renal outcome in ASVV. In other forms of glomerular disease, an elevated urine excretion of high molecular weight proteins, measured as urine

IgM, is an early marker of poor outcome. The aim of this study was to investigate prognostic factors in ASVV with special emphasis on urine IgM.

Methods: A single-centre observational study, the prognostic significance of urinary IgM, was analyzed using univariate analysis and compared with other variables at diagnosis, such as age, serum albumin, serum creatinine, sex, ANCA specificity, and albuminuria using multivariate Cox's regression analysis. Results: 83 consecutive patients (49 males/34 females) with ASVV with renal involvement were enrolled and followed for 60 months. Patient survival at 1 and 5 years was 93% and 77% respectively, and the corresponding figures for renal survival, censored for death, were 84% and 76%. Univariate analysis found patient survival to be associated with age, male sex, serum creatinine, low serum albumin and high urine IgM. Renal survival was associated with serum creatinine, high urine albumin and IgM. Multivariate analysis indicated only age and urine IgM to be independent predictors of patient survival (OR = 11.2 and 4.4, respectively, $P < 0.01$). Urine IgM was the only independent predictor of end stage renal disease (ESRD) (OR = 19.8, $P = 0.004$). Overall 35% of the patients reached the composite end point of either death or renal replacement therapy. Urine IgM excretion was the most potent predictor of such outcome (OR = 7.7, $P = 0.000$).

Conclusion: The occurrence of high amounts of IgM in urine at presentation is a strong marker for poor prognosis in patients with ANCA associated renal vasculitis.

C-Reactive Protein (CRP) as a Predictor of Relapse in Wegener's Granulomatosis (WG)

W. Schmitt, D. Münch, E. Csernok, J. Brade, W.L. Gross, F.J. van der Woude, B. Hellmich

University Clinic Mannheim, 5 Medizin, Mannheim, Germany

Background: Relapses in WG are common and result in accumulating morbidity. However, it is difficult to identify cases with an increased risk of relapse.

Objective: To assess the predictive power of a high-sensitivity (hs)CRP assay for subsequent relapse in WG patients in complete clinical remission.

Patients and Methods: Within the cohort of WG patients followed in a tertiary referral centre, all subjects were identified who were in complete clinical remission with follow up data available for at least 6 months and who did not suffer of coronary heart disease or generalised arteriosclerosis. Using a conventional and a hsCRP assay (Dade Behring), CRP levels were determined in all available serum samples of these subjects collected in the absence of infection between the onset of complete clinical remission and first relapse.

Results: Of 66 cases identified, 36 remained in complete remission during a mean follow up of 28.7 ± 9.5 months (123 sera available), whereas 30 relapsed after a mean follow up of 13.6 ± 9.5 months (68 sera available before relapse). Conventionally determined CRP levels and ESR did not differ between groups. In contrast, CRP levels retrospectively detected by the hsCRP assay were significantly higher in patients who later relapsed than in those who did not ($p = 0.004$; hsCRP 3.4 mg/l vs. 5.3 mg/l; no significant increase over time; area under the

curve (AUC) hsCRP = 0.63). Also, C-ANCA titers were higher in patients with a subsequent relapse (median titer 1:256 vs. 1:64; $p = 0.002$; AUC 0.64). At the time of relapse, hsCRP levels further increased (41.8 ± 24.6 mg/l), whereas C-ANCA titers did not (1:256).

Conclusion: During phases of complete clinical remission, hsCRP, but not conventionally determined CRP levels were found to be higher in WG patients with a subsequent relapse compared to those who remained in remission. The hsCRP assay may allow to detect subclinical levels of inflammation that are associated with an increased risk of relapse.

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Accuracy of Histological Findings of Small-vessel Disease to Distinguish Microscopic Polyangiitis (MPA) from Classic Polyarteritis Nodosa (c-PAN)

A. Mahr, O. Decaux, J. Authier, J.C. Jennette, K. Holl-Ulrich, L. Guillevin

Hospital Cochin, Internal Medicine, Paris, France

Background: MPA and c-PAN can be virtually indistinguishable based on clinical criteria. Conceptually, because of its small-vessel tropism, MPA is a vasculitis distinct from c-PAN, but it remains unknown if this histological criterion can be routinely used to assign a definite diagnosis.

Objective: To determine whether histological proof of small-vessel vasculitis (SVV) can reliably differentiate between MPA and c-PAN.

Methods: Two experienced pathologists, blinded to the clinical diagnoses, separately reviewed 27 vasculitis-positive neuromuscular ($n = 19$) and skin biopsies ($n = 8$), looking specifically for SVV. These specimens had been obtained from 26 selected patients with definite clinical diagnoses of MPA ($n = 14$) or active hepatitis B virus (HBV)-related c-PAN ($n = 12$). MPA was defined based on positive P-ANCA/anti-MPO or C-ANCA/anti-PR3 status and no features suggestive of Wegener's granulomatosis or Churg-Strauss syndrome; 64% of MPA patients had biopsy-proven pauci-immune glomerulonephritis.

Results: SVV was observed in 57% and 50% (pathologist 1 and 2, respectively) of MPA specimens, and in 46% and 69% of c-PAN samples. In c-PAN samples, SVV was seen in 22% and 56% of neuromuscular and 100% (by both pathologists) of skin biopsies. The inter-observer agreement (kappa coefficient) for histologically detected SVV was 0.25 (95% CI: 0.10 to 0.60).

Conclusion: The findings of this study suggest that at least for neuromuscular or skin biopsies, SVV is neither a constant lesion in MPA nor an element clearly discriminating MPA from c-PAN. For patients with uncharacteristic features compatible with both vasculitides, our results indicate that accurate classification based on histology can be difficult. Further investigation is warranted to determine whether these findings remain valid for c-PAN unrelated to HBV.

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Unusual Involvement of the Central Nervous System by Polyarteritis Nodosa

C. Perez, D. Echeverria, P. Fanlo, I. Elejalde, J.M. Arejola, V. Jarne, M. Muniesa

Department of Internal Medicine, Virgen del Camino Hospital, Pamplona, Spain

Background: Polyarteritis nodosa (PAN) is a multisystem, necrotizing vasculitis of small and medium-sized muscular arteries. Virtually all series are in agreement that central nervous system (CNS) dysfunction is less frequent than peripheral nervous system disease, is relatively uncommon at onset, and is virtually never the sole presenting feature of PAN.

Objective: To describe three patients with PAN who presented an unusual involvement of the CNS.

Patients and methods: Patient 1. A 61-year-old woman developed fever, hypertension, headache, coma, and a rash on the lower limbs. A skin biopsy disclosed signs of vasculitis. She had an inflammatory cerebrospinal fluid (CSF). Cultures of blood and CSF were negative. MRI of the brain showed multiple brainstem and periventricular lesions on T2-weighted images. There was a rapid response of her symptoms to steroid and cyclophosphamide therapy. Patient 2. A 54-year-old man presented with fever, headache, weight loss of 10 kg, myalgia, and tenderness of the leg muscles. Examination showed a peripheral left facial palsy, and bilateral papilledema. The lumbar puncture yielded inflammatory CSF, with a pressure of 280 mm. Cultures of blood and CSF were negative. MRI of the brain was normal. A muscle biopsy was negative. Visceral angiography showed multiple aneurysms of small and medium-sized arteries of the kidneys, liver, and intestine. He made a full recovery after treatment with steroids and cyclophosphamide. Patient 3. A 53-year-old woman developed arthralgia, fever, myalgia, headache, pain on the right face, weight loss, and cutaneous nodules on the lower limbs. T2-weighted MRI of the brain showed multiple small foci of hyperintensity in periventricular and deep white matter. A skin biopsy showed signs of vasculitis. She was treated with steroid and cyclophosphamide.

Conclusion: Acute encephalopathy, intracranial hypertension with facial palsy, and leucoencephalopathy should be considered as part of the neurological manifestations of PAN.

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Cutaneous Vasculitis during Selective Serotonin Reuptake Inhibitor Therapy

L.F. Flores-Suarez, C. Chanussot, M.E. Vega

Instituto Nacional de Nutricion, Immunology and Rheumatology, Mexico, D.F., Mexico

Objective: To present a case of cutaneous leukocytoclastic vasculitis in a patient treated with escitalopram. A 13-year-old girl presented with disseminated purpuric macules in both lower limbs after two-month treatment with escitalopram for depression. No other signs or symptoms were present. The biopsy disclosed an eosinophilic leukocytoclastic vasculitis with negative immune complex, C3, C4 or

IgA deposition. All other tests including ANCA, blood cell count and urinalysis were negative or normal. Escitalopram was stopped and the lesions resolved in one week. On accidental rechallenge 3 weeks later, the lesions reappeared and again disappeared after halting medication. She is currently treated only with psychotherapy. Discussion: Selective serotonin reuptake inhibitors comprise a new effective class of antidepressants which are widely prescribed. Citalopram has high affinity for antiserotonin receptors and its S-enantiomer, escitalopram is the newest drug of its kind. Side effects are mostly gastrointestinal, headache, agitation, restlessness and sexual dysfunction with other less common being bleeding, cardiac arrhythmias and the 'serotonin' syndrome. Acute urticaria and a benign maculopapular rash have been described as cutaneous reactions. Previously, only one case of cutaneous vasculitis associated with citalopram has been reported in the French literature. Our case seems clearly associated with escitalopram intake due to rechallenge confirmation and the finding of infiltrating eosinophils and negative immunofluorescence in the skin biopsy, features of a drug-induced vasculitis.

Conclusion: Postmarketing surveillance of this effective antidepressant should include the reporting of cutaneous vasculitis.

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Causes of Cutaneous Vasculitides (CV) in a General Hospital Setting Using a Systematic Approach: A One-year Incident Study

L.F. Flores-Suarez, C. Chanussot, M.E. Vega, J. Cabiedes, E. Reyes

Instituto Nacional de Nutrición, Immunology and Rheumatology, Mexico, D.F., Mexico

Objective: To search the cause of CV in a general setting during a one-year period and to increase the diagnostic yield which in the past was 25%.

Material and methods: Children or adults with CV attending a general hospital were prospectively evaluated with complete clinical examination, CBC, ESR, urinalysis, guaiac stool test, serum creatinine, PT, PTT, serum IgA, ANCA, MPO and PR3-ANCA, ANA, RF, cryoglobulins, antiphospholipid antibodies (IgG, IgA, IgM), C3, C4, hepatitis B and C serology. All patients had skin biopsy; in the majority direct immunofluorescence was done. ACR and Chapel Hill Consensus Conference criteria (CHCC) were used for classification. Final diagnosis were established by consensus between observers after completion of the diagnostic work-up.

Results: Five children and 20 adults were studied. There was female predominance (84% vs. 16%). In children, 1 had Schönlein-Henoch purpura (SHP), 2 had drug-associated CV; in adults 1 had polyarteritis nodosa, 1-microscopic polyangiitis (MPA), 1-thrombotic vasculitis (puerperal), 1-antiphospholipid syndrome, 2-systemic lupus erythematosus (SLE), 3-SHP, 2-paraneoplastic. Using ACR criteria 14/25 patients were classified (plus 2 who had SLE, total 16 patients). The rest did not satisfy criteria or there were no criteria for the disease (MPA). There was criteria overlap between SHP and hypersensitivity vasculitis (HSV) in 12 cases. 50% of them had either

one or the other, the rest were false positive (5 not classified-NC, 1 paraneoplastic). Using the CHCC 14/25 were classified with no diagnosis overlap; in the 10 patients with leukocytoclastic angiitis (LA) 2 had this diagnosis, the rest were false positive (1 paraneoplastic, 2 SHP, 5 NC). No patient had cryoglobulinaemia nor positive hepatitis serology. Overall, 56% could be classified. Discussion: With this work-up, the diagnostic yield increased twofold. However, in the case of HSV or LA and SHP none of the criteria proposed had satisfactory specificity; other parameters were used to discern between both. Six patients remained as NC. In our view, cryoglobulins and hepatitis serology do not seem useful initially unless patients' history supports they need to be done. Unclassified patients are being followed-up closely.

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Demographic Data in ANCA Associated Vasculitis

O. Floßmann, D.R.W. Jayne

Addenbrookes Hospital, Nephrology, Cambridge, England

Introduction: Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are both primary small vessel vasculitides which are associated with anti neutrophil cytoplasmic antibodies (ANCA). As they have a similar clinical presentation and response to therapy, they are often grouped together as ANCA associated vasculitides. The Objective is to report on baseline data of the first wave trials of the European Vasculitis Study Group (EUVAS).

Methods: Baseline data of three randomized prospective therapeutic trials, NORAM (n = 95), CYCAZAREM (n = 155) and MEPEX (n = 137) were included. An analysis of the baseline data on sex, age, diagnosis at study entry was performed. In addition a comparison of two disease scoring systems, the Birmingham Vasculitis Activity Score (BVAS) and the Disease Extend Index (DEI) was made. The calculated glomerular filtration rate (GFR) was analysed. A comparison of the organ involvement at presentation was performed between WG and MPA.

Results: There were 224 patients with WG and 161 with MPA. For 2 patients no diagnosis was recorded. The mean ($\pm$ SD) age of patients with WG was 54.4 ± 14.4 years compared with 61.8 ± 12.7 for patients with MPA ($p < 0.001$). The mean DEI was higher in WG 7.06 ± 2.70 than in MPA 5.38 ± 2.56 ($p < 0.001$). The BVAS was also higher in WG 17.86 ± 9.28 than in MPA 15.67 ± 8.31 ($p = 0.028$). ENT (77.2% vs. 22.8%), eye (43.6% vs. 15.2%) and nervous system (24.3% vs. 12.9%) involvement are more common in WG than in MPA, whereas renal (68.8% vs. 96.2%) involvement is more common in MPA. Increasing age was associated with a lower GFR (Correlation $r = -0.505$, $p < 0.001$). Patients with MPA have a lower GFR 17.33 ± 17.36 ml/min at presentation than patients with WG 39.26 ± 32.57 ml/min ($p < 0.001$).

Conclusion: The data of the trial indicate that vasculitis becomes more common with increasing age. Patients with WG are on average younger than patients with MPA.

Incidence of Primary Renal Vasculitis in Miyazaki, Japan

N.T.S. Fujimoto, S. Uezono, S. Hisanaga, K. Fukudome, S. Kobayashi, K. Suzuki, H. Hashimoto, H. Nuno

Juntendo University School of Medicine, Rheumatology, Tokyo, Japan

Background: Recently, an increasing incidence and recognition of primary renal vasculitis (PRV) seems to be evident since the relationship between the antineutrophil cytoplasmic antibody (ANCA) and rapidly progressive glomerulonephritis has been known. However, at population level, little is known about epidemiology of PRV in Asia area. In this study, we estimate the incidence of PRV in Miyazaki, Japan, through 2000 to 2004, retrospectively.

Methods: Patients with PRV have been included in this study by the following criteria in according with EUVAS (European Systemic Vasculitis Study Group); who 1) was a new patient with primary systemic vasculitis, 2) had renal and/or other organ involvements attributable to active primary systemic vasculitis, 3) was ANCA-positive, if the disease is not confirmed histologically. All patients received serology tests for ANCA and anti-glomerular basement membrane antibody.

Results: The registered number of the patients with PRV was 9, 9, 9, 17, and 13 in 2000, 20001, 2002, 2003, and 2004, respectively. The total number was 57 patients during these 5 years. Male and female was 25 vs. 32. The average age was 70.3 ± 10.8 years old and 79 percent of the patients were more than 65 years old. All the patients except one were from 3 areas in Miyazaki. The adult (>15 years old) population of all the three areas are 767,988. By these figures, the annual incidence of PRV was estimated as 14.8/million (95% C.I. 10.8–18.9). The incidence in these 2 years increased was 19.5/million/year. Ninety-one percent of the patients were MPO-ANCA positive, but nobody was PR3-ANCA positive.

Conclusion: The incidence of PRV in Japan was almost same as recent European estimates. However, almost patients had MPO-ANCA associated microscopic polyangiitis including renal limited vasculitis. The apparent difference in serology of PRV between Europe and Japan is necessary to clarified with further prospective, international collaborative studies.

Prevalence of Microscopic Polyangiitis/Wegener's Granulomatosis and the Ratio of MPO, PR-3- ANCA in Patients with ANCA-associated Vasculitis in Japan

S. Kobayashi^{1,2}, N. Tamura², T. Ihara³, E. Muso⁴, K. Suzuki⁵, M. Yoshida⁶, K. Nakabayashi⁷, N. Tsuchiya⁸, M. Kurosawa⁹, Y. Inaba⁹, S. Fujimoto¹⁰, H. Nuno¹¹, H. Hashimoto^{1,2}

Internal Medicine, Juntendo Koshigaya Hospital¹, Rheumatology, Juntendo University, School of Medicine², Internal Medicine, Kyoto University, School of Medicine³, Nephrology, Kitano Hospital⁴, Bioactive Molecules, National Inst. of Infectious Disease⁵, Nephrology, Hachioji Medical Center, Tokyo Medical School⁶, First Department of Internal Medicine, Kyorin University, School of Medicine⁷, Human Genetics, Graduate School of Medicine, University of Tokyo⁸, Hygiene and Epidemiology, Juntendo University⁹, First Department of Internal Medicine, Miyazaki Medical College, Miyazaki University¹⁰, Pediatrics, Miyazaki Medical College, Miyazaki University¹¹

Objective: Incidence of primary ANCA-associated vasculitides (AAV) and type of ANCA?P-,MPO-/C-,PR-3-? has been reported to be different between the Northern and the Southern part of European countries. In Japan (located at the latitude of 30–45°N), the prevalence of microscopic polyangiitis (MPA) is much higher than that of Wegener's granulomatosis (WG). To elucidate epidemiological characteristics of AAV in Japan, prevalence of MPA and WG, the rate of positive for p-,MPO-/c-,PR-3-,ANCA, and HLA genotyping in patients with AAV were studied.

Methods: The prevalence of AAV and the rate of positive for p-/c-ANCA were analyzed by a nationwide hospital-based survey which was held in 1994 and 1998 by the Research Committee of Intractable Vasculitides and the Research Committee of Epidemiology, the Ministry of Health and Welfare of Japan. Genotyping was performed including HLA-DRB1 typing in 69 patients including 50 MPA patients.

Results: In 1994, the estimated number of patients with polyarteritis nodosa/PN was 1,400, the average age was 56.2 and male/female ratio was 1:1.1. The estimated number of patients with WG was 670, the average age was 46.2 and male/female ratio was 1:1.2. In 1998, 63 patients with MPA, 28 with WG, 12 with CCS and 104 with renal limited AAV were reported. Prevalence, estimated patients treated in 1997, of WG was 2.0/million, CSS was 0.8/million and MPA was 15.0/million. p-/MPO-ANCA were demonstrated in 87.3% and 86.5% patients with MPA and renal limited AAV. c-/PR-3 ANCA were demonstrated in 85.7% patients with WG. A significant association of HLA-DRB1*0901 with MPA ($p = 0.0037$), as well as p-/MPO-ANCA positivity ($p = 0.0014$) was detected (J Rheumatol. 2003;30: 1534–40).

Discussion: The prevalence of AAV in Japan was determined by nationwide, hospital based, retrospective study in 1998. The prevalence of WG was 2.0/million and MPA was 15.0/million, which were lower than those reported from other countries. These results seem to

be due to the difference of the methods and incomplete understandings of AAV in the late '90 in Japan. The number of patients with MPA is approximately 8 times more than that of WG. This result makes sense since we see very few patients with WG in Japan. HLA-DRB1*0901 is one of the most frequent alleles in the general Asian population (5–17%), but it is rare in other populations (0–3%) This may be one of the reasons of epidemiological difference of AAV between Europe/USA and Japan. Recently, further investigation has set and started to determine the incidence of AAV by population-based, prospective study in Miyazaki and Okinawa, Japan.

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Three Different Subsets of Pulmonary-renal Syndrome Associated with MPO-ANCA, Anti-GBM Antibody or Immune Complexes and their Clinicopathologic Features

K. Nakabayashi, K. Fukuoka, Y. Arimura, A. Yamada, T. Nagasawa

First Department of Internal Medicine, Kyorin University School of Medicine, Japan

Objective: Twenty-four cases with pulmonary-renal syndrome (PRS) were categorized into 4 subsets which were based on MPO-ANCA, anti-GBM antibody (AÆGBM Ab), or circulating immune complexes (CIC). These subsets were studied for clinical and pathological features.

Methods: MPO-ANCA and AÆGBM Ab were detected by EIA. Thirteen cases belonged to MPO-ANCA positive group, 4 to AÆGBM Ab positive group, 3 to both MPO-ANCA and AÆGBM Ab positive group, and 4 to CIC group.

Results: female/male ratio, average age, serum creatinine (Cr) level, and the interval from illness onset to renal failure in the each group were as follows. MPO-ANCA positive group was 4/9, 71 years old, 5.3ÜJ/ÜO, and 26 weeks. AÆGBM Ab positive group was 2/2, 61 years old, 10.0ÜJ/ÜO, and 4 weeks. Both MPO-ANCA and AÆGBM Ab positive group were 2/1, 60 years old, 11.3ÜJ/ÜO, and 8 weeks. CIC group was 3/1, 43 years old, 1.5ÜJ/ÜO, and 3.5 weeks. Renal pathology showed different manifestations in the each group. MPO-ANCA positive group had crescents from partial to circumferential and tubulointerstitial (TI) lesions in all cases. AÆGBM Ab positive group and both MPO-ANCA and AÆGBM Ab positive group had circumferential crescents and TI lesions in all cases. CIC group had only partial crescents. IF study disclosed pauci-immune deposits in MPO-ANCA positive group, linear deposits in AÆGBM Ab positive group and in both MPO-ANCA and AÆGBM Ab positive group, and granular deposits for IgG and C3 in CIC group.

Conclusion: MPO-ANCA positive group were old-aged patients and showed smoldering progression of illness, not rapid progression, in the half of cases. In addition, the magnitude and percentage of crescents varied in the each case. AÆGBM Ab positive group demonstrated to become to renal failure within 4 weeks from the onset of illness and circumferential crescents. Both MPO-ANCA and AÆGBM Ab positive group fell to renal failure in 8 weeks and revealed circumferential crescents. CIC group had not renal failure and showed only partial crescents. These different features suggest to treat each group differently.

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Otorhinolaryngological Involvement in a Group of Latin American Patients Wegener Granulomatosis

L.F. Flores-Suarez, O. Beltran

Instituto Nacional de Nutricion, Immunology and Rheumatology, Mexico, D.F., Mexico

Objective: To describe the relevant otorhinolaryngological (ORL) findings in a cohort of patients with Wegener granulomatosis (WG) attending a tertiary referral centre.

Methods: Retrospective chart review (1978–2005). The following variables were obtained: gender, age, initial disease manifestations, span between their appearance and definitive diagnosis of WG and evolution of ORL involvement. These were subclassified according to areas.

Results: 64 WG patients, 29 women and 35 men with a mean age of 41 ± 14 years: 16% with localised, 84% with generalised disease. Mean time between initial symptoms and diagnosis-36 months. At the beginning of the disease 80% ($n = 51$) had ORL symptoms which increased throughout evolution to 95% ($n = 61$). Only 3 have not had ORL compromise. By areas, the nose was the most frequently affected (84%) with crusting (63%), epistaxis (56%) and obstruction (55%) as main symptoms; saddle nose deformity was seen in 17%. Nasal biopsies were obtained from 22 patients. In 60% the results confirmed active WG. Sinusitis was present in 65%, and in most cases the manifestations responded favourably to antibiotic treatment. Only 6 patients required surgical drainage and from these 1 had histological confirmation of activity. Laryngotracheal symptoms: dysphonia-30%, dyspnea-19%, stridor and subglottic stenosis-16%, tracheal stenosis-6%, bronchial stenosis-5%. Four patients (6%) have permanent tracheostomy. Ear involvement: neurosensorial deafness-28%, medial otitis-23%. 15% had oral ulcers attributed to active disease.

Conclusion: ORL involvement is the most frequent in our patient's population, both at the start of disease and during its evolution. Therefore, patients seek ENT attention frequently at onset, the role of the ENT specialist being of utmost importance. Most ORL symptoms are nasal and a good clinical evaluation with prompt histological confirmation can be obtained. The positive yield of WG histological findings in nasal biopsies is similar in our centre (60%) to that from centres specialised in WG care (70%) and higher when compared to reports from other series (25%).

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Disease Extent Index in Takayasu Arteriitis

S. Rajappa

9/2 Rajarathinam Street, Kilpauk, Chennai, India

Background: Takayasu aorto-arteritis is a rare form of 10SV that appears to be commoner in Asia than Europe or N America. The restricted distribution of vessel involvement leads to a pattern of disease that differs markedly from the well-documented small/medium vessel vasculitides. Thus assessments of disease activity or severity that have been used productively to document therapy response and

outcome in AA.SV are not helpful in Takayasu. The IRAVAS group therefore set out to devise an index of disease severity and extent that could be useful in studying this disease.

Methods: The well-established activity index BVAS was taken as a template for the initial discussions among an expert group with clinical experience of the condition, aimed at producing a consensus on necessary items. This was then applied to limited numbers of patients and rehoned by further group discussions, aiming for inclusivity at this stage in order not to omit important but infrequent items. The draft produced was then applied to a large group of patients seen at 2 centres. The data from this was used to further trim the draft index by omitting unused items that were agreed to be unnecessary while further emphasising key factors seen frequently.

Results: 143 patients, mean age 38.8 (± 13.5), sex ratio 1.5:1 female preponderance, from N and S India were studied. Systems scored commonly included systemic (59 patients), renal (62), and CVS (88). The latter focussed on pulse abnormalities, including pulse loss (40), pulse inequalities (42), and bruits (22). Ischaemia evidenced by claudication was seen in 22. Aspects of heart involvement included aortic incompetence, angina, and CCF (10 each). Systems rarely or never used included skin, mucous membranes, and abdominal. No further significant items were uncovered through the "Other" box, despite emphasising its importance to this exercise. The final format derived from this contains 59 items in 11 organ-based systems. The most important system, CVS, contains 19 items, with special emphasis on bruits, pulse loss and claudication. A glossary has been produced to aid standardisation of application. At this stage all positive items are included, irrespective of whether they are new or old, but the duration of involvement is also noted.

Conclusion: The current index is in a usable size and format and this is now being applied to collect data on the pattern of aortoarteritis in collaborating clinics across India. With further experience and a larger data set, additional minor modification may be required. However the current version is a practical tool which will be useful for studying important aspects of aorto-arteritis, such as differences in anatomical distribution between series/countries; individual disease severity; and outcome. It should also find use as a clinical tool to compare with newer imaging techniques to assess disease involvement such as MR and PET. We hope this will be a first step to producing a practical clinical index of disease activity for use in areas where access to expensive new imaging is very restricted.

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MRI Vessel Wall Imaging in Giant Cell Arteritis: Assessment of Mural Inflammation, Determination of the Cranial Involvement Pattern and Non-invasive Monitoring under Corticosteroid Therapy

T.A. Bley, M. Markl, M. Uhl, K. Warnatz, P. Vaith, D. Schmidt, H.H. Peter, M. Langer

University of Freiburg, Diagnostic Radiology, Freiburg, Germany

Purpose: The aim of this study was to investigate the value of high resolution MRI for cranial, cervical and thoracic vessel wall imaging in GCA.

Methods: 67 consecutive patients with clinically suspected GCA underwent high resolution MRI with an sub-millimeter spatial resolution of 0.2 mm $\times$ 0.3 mm. Signs of mural inflammation such as increased signal intensity from contrast enhancement and thickening of the wall were judged according to a 4 point ranking scale. MRI diagnosis was then compared to the final rheumatological diagnosis including temporal artery biopsy in 32 cases. In 21 patients, the involvement pattern of mural inflammatory changes of all major superficial cranial arteries was investigated. MRI was repeated after 8 to 15 months of corticosteroid medication to evaluate treatment response.

Results: In all cases, the superficial cranial arteries could be depicted in good diagnostic quality. Sensitivity and Specificity of MRI were 83.3% and 97%, respectively. The positive predictive value and the negative predictive value of MRI were 96.2% and 86.5%, respectively. In all GCA patients multiple cranial arteries showed MRI signs of inflammation. In one patient, the occipital arteries were inflamed while the temporal arteries were spared. Mural inflammation decreased significantly in all but one patient under corticosteroid medication. One patient presented with inflammatory stenoses in both subclavian arteries. Discussion This study conforms the capabilities of high resolution MRI to depict the inflammation of relatively small vessels in GCA. MRI visualized inflammation of the superficial cranial arteries allowing for diagnosis with good sensitivity and specificity as conformed with the criteria of the ACR. It may be used to monitor the activity of mural inflammatory changes under long term corticosteroid therapy. In summary, this technique might be useful for non-invasively deriving the correct diagnosis, evaluating severity of the disease, and monitoring therapy, severity of renal impairment increases with advancing age and is worse in MPA.

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Positron Emission Tomography in the Diagnosis and Therapy of Aortoarteritis

R. Becvár, P. Fencl, V. Cerníková

Rheumatology Institute of 1st University Faculty UK, Praha 2, Czech Republic

Early diagnosis of aortoarteritis remains still a problem as symptoms and laboratory tests are nonspecific. X-ray angiography or magnetic resonance imaging are still considered as 'gold standard'. We describe five female patients in which the diagnosis of aortoarteritis was set by positron emission tomography (PET) and in three of them later confirmed by x-ray angiography. The repeated PET scans during the therapy have shown a decrease of glucose uptake which correlated with clinical improvement and acute phase reactants changes. We may assume that PET may represent an additional useful information towards the diagnosis and management of aortoarteritis.

Magnetic Resonance Imaging Detects Myocardial Damage in Churg-Strauss Syndrome

U. Goebel, R. Wassmuth, J. Schulz-Menger, A. Natusch, W. Schneider, R. Kettritz, F.C. Luft

Helois Kliniken Berlin, 1. Innere/FVK, Berlin, Germany

Objective: We report on the use of cardiac magnetic resonance imaging (MRI) in patients with Churg-Strauss syndrome-related heart disease. A myocarditis and cardiomyopathy may result. MRI precisely quantifies left ventricular function and additionally depicts reversible and irreversible tissue damage.

Methods: We studied ten consecutive patients (eight women, age 27 to 70 years) with Churg-Strauss syndrome suspicious for cardiac involvement with cardiac magnetic resonance imaging. All patients had asthma, eosinophilia and various organ manifestations including skin, nervous system and arthralgia. 5 out of 10 patients were ANCA-positive. All fulfilled the Chapel Hill Consensus Conference criteria. Cardiac symptoms included heart failure, palpitations and malignant arrhythmias. Coronary angiography was available in 5 patients. Magnetic resonance cine imaging, T2-weighted fast spin echo edema imaging, contrast enhanced T1-weighted fast spin echo and delayed enhancement imaging were performed on a 1.5 T MRI scanner. All patients underwent biopsy. Median MRI follow-up was 11 months.

Results: MRI studies were pathologic in all but one case. At initial diagnosis, mean ejection fraction was 49% (14–68%). Increased left ventricular enddiastolic volume and mitral regurgitation were found in four out of ten patients. Five patients had pericardial effusion. Myocardial edema was present in 3/10 patients. Early contrast uptake as a marker of increased interstitial space was observed in 5 patients. Seven patients had delayed contrast enhancement consistent with irreversible myocardial damage persisting during follow-up. These lesions were located in the subendocardium or in the middle of the myocardial wall and were confirmed by biopsy despite normal coronary arteries. In two cases pathological contrast uptake was found although left ventricular function was still preserved.

Conclusion: Cardiac magnetic resonance imaging depicts various stages of myocardial damage in Churg-Strauss-syndrome beyond traditional markers of left ventricular function.

Histopathologic Findings in Temporal Artery Biopsies from 171 Patients with Giant-cell Arteritis (GCA). Relationship with Prospectively Recorded Clinical Manifestations

J. Hernández-Rodríguez, I. Villar, A. García-Martínez, C. Font, M.J. Esteban, J.M. Grau, M.C. Cid

Hospital Clinic, Internal Medicine, Barcelona, Spain

Background: Temporal arteries from patients with GCA exhibit a variable extent of inflammatory involvement ranging from slight

adventitial infiltrates to full-developed granulomatous lesions involving the entire artery wall. The aim of our study was to investigate the relationship between the extent of inflammatory infiltrates in temporal artery biopsies and GCA-derived clinical manifestations.

Patients and Methods: Histopathologic examination of temporal artery biopsies from 171 patients (115 females and 56 males) with biopsy-proven GCA with prospectively recorded clinical manifestations including cranial symptoms, ischemic complications, polymyalgia rheumatica, constitutional symptoms (fever, weight loss, anemia) and duration of clinically apparent disease until diagnosis. 52 ± 39 H&E stained sections per biopsy were examined.

Results: Thirty-four (20%) specimens exhibited just adventitial infiltrates (adventitial pattern) and 137 (80%) displayed inflammatory infiltrates extending through the entire artery wall (panarteritic pattern). 117 of the later (85%) had granuloma formation, 91 of them (66%) with giant-cells. Patients disclosing the panarteritic pattern had higher frequency of cranial symptoms (88% vs 68%, $p = 0.005$); among them, headache (78% vs 59%, $p = 0.021$), scalp tenderness (53% vs 23.5%, $p = 0.002$), and jaw claudication (46% vs 26.5%, $p = 0.039$), than patients with inflammation restricted to the adventitia. The adventitial pattern was more frequent among patients with isolated PMR ($n = 12$) than in those with cranial symptoms (67% vs 17%, $p = 0.003$). No relationship was observed between the histopathologic extent and other variables considered including ischemic complications, fever, weight loss, or duration of cranial manifestations before the temporal artery excision.

Conclusions: In this large series of patients with GCA, panarteritic involvement of epicranial arteries appears to correlate with prominent cranial symptoms whereas systemic manifestations or ischemic events are not related to the extent of vascular inflammation in the samples examined.

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Sustained Spontaneous Remission in Giant-Cell Arteritis (GCA). Report of 2 Patients with Extended Follow-up

J. Hernández-Rodríguez, A. García-Martínez, J.M. Grau, M.C. Cid

Hospital Clinic, Internal Medicine, Barcelona, Spain

Background: Patients with GCA experience a dramatic relief of their symptoms with corticosteroid treatment. However disease outcome is highly variable among patients. While some patients endure sustained remissions with relatively short treatment periods other suffer from a relapsing disease requiring remarkable cumulated corticosteroid doses with their ensuing adverse effects. We illustrate this heterogeneity by describing 2 patients with biopsy-proven GCA who underwent spontaneous, long-lasting remission with no treatment. Case reports: Patient 1 was a 90y-old man referred by his primary care physician because of enlarged, pulseless temporal arteries with elevated ESR (70 mm). He was totally asymptomatic but his medical past history revealed that 2 years earlier he had experienced malaise and weight loss for several weeks and suffered one episode of amaurosis fugax. His complaints resolved spontaneously. Temporal artery

biopsy revealed GCA (healing pattern). He remained asymptomatic and died from pneumonia at age 94. Patient 2 was a 62 year old man referred because of malaise, weight loss, and elevated ESR (110 mm) which started 4 months earlier when he had an extensive work-up to rule out malignancy. His symptoms progressively improved. At evaluation, he was asymptomatic and but referred mild, self-limiting, past jaw claudication and his left temporal artery had reduced pulse. His ESR was 69 at that time. Temporal artery biopsy showed inflammation in small vessels surrounding the temporal artery extending towards the adventitia with remarkable intimal hyperplasia. He was asymptomatic at that time and ESR progressively went back to normal (9 mm) with no treatment. Two years later, a right temporal artery biopsy showed GCA features (healing pattern). His subsequent follow up was uneventful. He is alive and well 10 years later.

Conclusion: These 2 cases illustrate that GCA may be a self-limiting disorder and underline the highly variable and unpredictable outcome of the inflammatory process in GCA.

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Takayasu Arteritis Associated with Ulcerative Colitis-case Report and Review of the 66 Japanese Cases

T. Yamato

Kyorin University School of Medicine,
Internal Medicine, Japan

Takayasu Arteritis (TA) Associated with Ulcerative Colitis (UC) - case report and review of the 66 Japanese cases- T. Yamato, Y. Arimura, K. Nakabayashi, A. Yamada First Department of Internal Medicine, Kyorin University School of Medicine, Tokyo, Japan A 26-year-old male with 8 years history of UC was admitted because of diarrhea and right side neck pain in October 2000. He was treated by 30 mg/day of prednisolone, which improved his symptoms. As prednisolone was tapered, his neck pain worsened again accompanied by cervical vascular murmur. MRI showed bilateral carotid arteries increased in wall thickness and decreased luminal diameter in the right side. Angiography showed stenosis of the bilateral subclavian arteries, stenosis and poststenotic dilatation of the descending thoracic aorta, and irregularity of the abdominal aorta. According to ACR criteria he was diagnosed as TA associated with UC. We reviewed 66 Japanese cases of TA associated with UC reported from 1964 to 2004. Male to female ratio was 1:2.3. TA symptoms appeared at the age of 9 to 43 (23.2 Å 8.2). In 68.2% cases, UC preceded TA. In 4.5%, symptoms of UC and TA developed simultaneously. Time lag between the onset of UC and TA was 6.1 Å 5.4 years. Main types of affected vessels were Type I (38.9%) and Type V (24.1%). TA types were classified according to the Japan Ministry of Health and Welfare criteria. In HLA analysis, B52 was positive in 78.4% (A24:56.8%, DR2:62.2%). 18.2% of the patients had received cardiovascular system operations. ANCA was studied in 3 cases, and was detected

only in our case. In conclusion, in Japan, TA associated with UC is not rare. The typical features were young adult females with Type I vascular involvement, positive HLA B52 and developing subsequent to UC.

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Treatment of Anca-associated Vasculitides: Corticosteroids and Pulse Cyclophosphamide Followed by Maintenance Therapy with Methotrexate or Azathioprine: A Prospective Multicenter Randomized Trial (Wegent)

A. Mahr, C. Pagnoux, P. Cohen, M. Hamidou, M. Ruivard, X. Puéchal, L. Mouthon, L. Guillevin, for the French Vasculitis Study Group

Hospital Cochin, Internal Medicine, Paris, France

Background: Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are ANCA-associated vasculitides affecting small-sized vessels. After successful induction therapy with corticosteroids (CS) and cyclophosphamide (CYC), remission can be effectively maintained with azathioprine (AZA).

Objectives and Methods: We conducted a prospective multicenter randomized trial to study the efficacy and safety of treating WG and MPA with CS and pulse IV CYC, then randomized maintenance therapy with AZA or methotrexate (MTX), for patients with newly diagnosed WG or MPA. Induction therapy was IV CYC (0.7 g/m² on days 0–15–30, then every 3 wk until remission, followed by 3 additional pulses) and oral CS. Thereafter, patients were randomized to receive 12 months of maintenance therapy with oral AZA (2 mg/kg/d) or MTX (0.3 mg/kg/wk).

Results: 201 patients were assessed for eligibility between March 1998 and January 2004; 42 did not satisfy the inclusion criteria. Among the 159 eligible patients, 33 (21%) failed to respond to induction, then 12 were excluded from the final analysis because their maintenance therapy had largely exceeded 12 months for no justified reason. Ultimately, 114 patients were evaluated (86 WG, 28 MPA; BVAS at diagnosis 22 ± 7; cumulative CYC dose 10.4 ± 3 g): 55 were assigned to receive AZA and 59 to MTX. All the patient characteristics evaluated were comparable at randomization. For mean respective follow-ups since diagnosis and randomization of 36.8 ± 14 and 28.6 ± 13 mo, no significant differences were observed between both arms in terms of severe adverse events requiring assigned-maintenance withdrawal (13% vs. 20%, P = 0.28). Three MTX and 1 AZA recipients died (P = 0.27). Relapse-free survival rates at 18 and 36 mo post-diagnosis were, respectively: 87% [95% CI: 78–96] and 59% [44–74] for AZA arm vs 90% [82–98] and 67% [53–81] for MTX arm (P = 0.33).

Conclusion: Our results suggest that CS and IV CYC, followed by maintenance with AZA or MTX is an effective therapeutic strategy. However, although not statistically significant, MTX appeared to be associated with more frequent adverse events.

Randomised Controlled Trial of Daily Oral versus Pulse Cyclophosphamide for Induction of Remission in ANCA-associated Systemic Vasculitis

K. de Groot, D.R.W. Jayne, V. Tesar, C.O.S. Savage for EUVAS

Medical School Hannover, Nephrology, Hannover, Germany

Objective: The standard therapy of systemic ANCA-associated systemic vasculitis (AASV) with cyclophosphamide (CYC) and prednisolone is limited by its toxicity. This prospective, randomized, controlled trial aimed to determine whether oral daily CYC could be replaced by pulse CYC, using a lower cumulative CYC dose, for induction of remission in systemic AASV.

Methods: Patients with newly diagnosed systemic AASV and renal involvement (serum creatinine 150–500 $\mu\text{mol/l}$), and without immediate life-threatening organ manifestations were randomized to either a consensus standard regimen of daily oral CYC, 2 mg/kg/day or a pulse CYC regimen (a two week interval between the first 3 pulses then a three week interval), both with the same prednisolone regimen. Three months after achievement of remission CYC was replaced by azathioprine in both groups, patients were followed until month 18. The primary endpoint was the disease free period (DFP) between remission and first relapse or study end.

Results: 160 patients were recruited. Preliminary results from 140 complete data sets did not reveal significant differences in DFP (pulse CYC 13.0 vs oral CYC 12.4 months, $p = 0.5$), time to remission and to relapse, or cumulative survival (pulse CYC 4/74 vs oral CYC 12/59 deaths, $p = 0.06$, Kaplan Meier analysis) between treatment groups. On an intention to treat basis, the cumulative CYC dose was twice in the daily oral CYC group as compared to the pulse group.

Conclusion: Pulse CYC was equally effective as standard daily oral CYC for induction of remission in systemic AAV with renal involvement with a significantly reduced cumulative CYC dose. Thus, pulse CYC is recommended as the regimen of choice for this indication.

Azathioprine as Compared to Cyclophosphamide Maintenance Therapy for ANCA-associated Vasculitis is Associated with Increased Long-term Relapse Risk

J.S.F. Sanders, P.M. Stassen, C.G.M. Kallenberg, C.A. Stegeman

University Medical Center Groningen, Clinical Immunology, Groningen, The Netherlands

Objective: In patients with ANCA-associated vasculitis relapses are frequent within 5 years after diagnosis. The CYCAZAREM study (NEJM 2003;349:36) showed that patients switched to azathioprine

after induction of remission with cyclophosphamide as compared to continuous cyclophosphamide did not have an increased risk of relapse at 18 months. We evaluated long-term disease-free survival in patients with ANCA-associated vasculitis on cyclophosphamide maintenance as compared to patients switched to azathioprine.

Methods: Patients diagnosed at our center with ANCA-associated vasculitis between January 1990 and April 2003 and at least one year follow-up were included ($n = 176$). From 1990 to 1996 new patients were treated with cyclophosphamide (2 mg/kg, tapered by 25 mg per 3 months). From 1997 patients were increasingly switched to azathioprine (1.5–2.0 mg/kg, tapered by 25 mg per 3 months) after 3 months of remission on cyclophosphamide. Actuarial disease-free survival was analysed by logrank test. Results Apart from age (56 ± 13.4 vs. 50 ± 18.8 ; $p = 0.017$) clinical characteristics at diagnosis did not differ between patients on cyclophosphamide ($n = 100$) and on azathioprine maintenance ($n = 76$). 126 patients were diagnosed Wegener's Granulomatosis, 34 Microscopic Polyangiitis and 16 Necrotizing Crescentic Glomerulonephritis. ANCA were detected against PR3 ($n = 132$), MPO ($n = 43$), and HLE ($n = 4$). At 18 months relapse-free survival was 88.0% in the cyclophosphamide and 86.7% in the azathioprine group. In contrast, at 5 years relapse-free survival was 59.0% in the cyclophosphamide and 37.7% in the azathioprine group (RR 1.53; 95% CI 1.02–2.47; $p = 0.042$). Both patients with MPO (RR 2.27 (0.56–9.09)) and with PR3-ANCA (RR 1.66 (1.05–2.80)) relapsed more frequently on azathioprine maintenance.

Conclusion: Although not from a randomized controlled trial our data indicate that patients with ANCA-associated vasculitis on azathioprine as compared to cyclophosphamide maintenance may have a significantly increased relapse risk on long-term follow-up.

Mycophenolate Mofetil in ANCA Associated Systemic Vasculitis

O. Flossmann, M. Koukoulaki, D.R.W. Jayne

Addenbrookes Hospital, Nephrology, Cambridge, England

Background: Mycophenolate mofetil (MMF) is an immune suppressive initially introduced for the prevention of solid organ allograft rejection that is increasingly used in autoimmune conditions, including vasculitis.

Methods: This retrospective study evaluated the efficacy and tolerability of MMF in 51 sequential patients with ANCA Associated Systemic Vasculitis (AASV) treated in a single centre between 2001 and 2004.

Results: The mean age was 54 years and median disease duration was 36 months. A mean of 3.5 systems were involved and the previous median exposure to cyclophosphamide was 9 grams. MMF was administered either as induction therapy (3/51, 6%), remission maintenance therapy (29/51, 56.9%), or as treatment for disease relapse (19/51, 37.5%). The mean duration of MMF therapy was 20 months and the mean MMF dose during the first year was 1.6 grams/day. 14/29 (48.3%) of those receiving MMF for remission maintenance therapy eventually relapsed with a mean time to relapse 14 months. Of those receiving MMF for relapsing disease, three failed to respond to therapy while the rest achieved remission by 3.9 months. However

nine of these subsequently flared; mean time to disease flare was also 14 months. MMF was withdrawn in 28 patients (54.9%) because of treatment inefficacy in 21, severe adverse events in five and intolerance in two. 36/51 (70.6%) experienced at least one side effect, namely infections in 24, gastrointestinal side effects in 12 and psychological events in six.

Conclusions: We have observed varying efficacy of MMF in AASV, with over 50% of patients with relapsing disease achieving remission and marked falls in concomitant steroid doses. However, longer follow-up indicates a subsequent relapse rate of over 50% that may be associated with low MMF dosing.

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Unexpected High Relapse-rate under Oral Methotrexate for Maintenance of Remission in Wegener's Granulomatosis. The LEM-trial Comparing Leflunomide versus Methotrexat

C. Metzler, N. Miehle, K. Manger, C. Iking-Konert, W.L. Gross, E. Reinhold-Keller for the German network of rheumatic diseases

University of Schleswig-Holstein, Campus Luebeck, Rheumatology, Luebeck, Germany

Introduction: In Wegener's granulomatosis (WG) methotrexate (MTX) and leflunomide (LEF) are used for maintenance of remission in uncontrolled studies. This study therefore compares the effectiveness and the safety of LEF and MTX.

Patients and Methods: In a multicentric, prospective randomised design patients with generalised WG in complete or partial remission after induction with CYC were treated alternatively with LEF 30 mg/d or MTX 20 mg/week orally over two years. Primary end point was the occurrence of a relapse. Secondary outcome parameters were DEI, BVAS, SF-36, cANCA-titre, ESR and CRP. Results: 54 patients were included in the study, 26 in the LEF-arm, 28 in the MTX-arm. In the LEF-group 6 patients suffered a relapse, thereof one major pulmonary relapse. In the MTX-arm 13 relapses were noted, seven major (four renal, two pulmonary and one central-nervous activity). The increased number of severe relapses in the MTX-group until 9/03 resulted in a premature termination of the study. The secondary outcome remained stable in the patients without a relapse and the concomitant PRD could be reduced those patients in both arms. In the LEF-arm 4 patients were withdrawn due to side effects: Hypertension (n = 2), peripheral neuropathy (n = 1) and leucopenia (n = 1).

Conclusion: The number of major relapses with this MTX-protocol is elevated in comparison to the LEF-protocol. In contrast, the side effect related withdrawals prevail clearly in the LEF-group. We see further need for controlled studies using different protocols (higher dosage of MTX with parenteral application), comparing further alternatives (mycophenolate mofetil, azathioprine).

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Therapeutic Effect of Anti-TNFalpha Antibodies in an Experimental Model of ANCA-associated Systemic Vasculitis

M.A. Little, G. Bhargal, J. Dangerfield, M. Nakada, H.T. Cook, S. Nourshargh, C.D. Pusey

Imperial College London, Renal Section, London, UK

The therapeutic options for ANCA-associated systemic vasculitis (AASV) remain limited and hampered by adverse effects. One potential novel therapeutic avenue involves inhibition of TNF α , with some encouraging uncontrolled data in humans with one agent (infliximab), but disappointing controlled data from another (etanercept). We investigated the therapeutic effect of a murine monoclonal anti-rat TNF α antibody (CNTO-1081) in a rat model of AASV (experimental autoimmune vasculitis, EAV). WKY rats immunised with human myeloperoxidase (hMPO) develop ANCA directed against rat MPO, pauci-immune crescentic nephritis and lung haemorrhage. The circulating ANCA in these rats enhance leukocyte adhesion and transmigration. On day 28 post-immunisation, a point when EAV is established, we randomised animals to treatment with CNTO or control mouse IgG (both 16 mg/kg) 3 doses/wk up to day 56. Treatment with CNTO reversed albuminuria (mean 1.1 ± 0.3 versus 8.0 ± 1.9 in controls, $p < 0.05$) and crescent formation (0% of CNTO group versus 60% of controls, $p < 0.05$). This was accompanied by a decline in ED1 positive glomerular macrophage infiltration (median 25, range 11–94 versus 83, range 43–156/50 glomeruli in controls, $p < 0.05$) and tubulo-interstitial nephritis (mean score 1.2 ± 0.2 in CNTO group versus 2.3 ± 0.3 in controls, $p < 0.05$). Lung haemorrhage was also reduced (median lung haemorrhage score 0, range 0–2 v 2, range 1–3 in controls, $p < 0.05$). When analysed by intravital microscopy, there was a 43% leukocyte transmigration block in response to topical CXCL1 (a neutrophil chemoattractant) in the CNTO group ($p < 0.001$). Anti-hMPO antibody titres were the same in both groups. We conclude that TNF α plays a role in the pathogenesis of EAV and that blockade of this cytokine with a monoclonal antibody is effective in treating established vasculitis.

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Pathways to Renal Biopsy among Patients with ANCA Small Vessel Vasculitis

C.E. Jennette, H. Chin, R.J. Falk, P.H. Nachman, S.L. Hogan

UNC Chapel Hill, Nephrology & Hypertension, Chapel Hill, USA

Renal manifestations of ANCA vasculitis occur in up to 75% of patients and significantly impact morbidity. Delay in diagnosis and treatment may contribute to poor renal outcomes due to the often rapidly progressive nature of ANCA glomerulonephritis. The purpose of this study was to evaluate pathways from onset of symptoms to renal biopsy among ANCA-SVV patients. Disease symptoms and number of physicians seen prior to renal biopsy were assessed by telephone interview in 129 patients with ANCA glomerulonephritis. For

patients, medical care sought in ≤ 1 month of symptoms defined direct and >1 month or >3 different physicians seen prior to a nephrologist defined a delay. For physicians, referral for renal evaluation in ≤ 1 month defined direct and >1 month defined delay. Comparisons were made between DIRECT and DELAYed access to nephrology care combining both patient and physician components. Median (range) is given for continuous measures and rank tests were used for comparisons. Patient delay in seeking any medical treatment (46%) and physician delay in referral to a nephrologist (74%) were common. Among patients who sought prompt care, 73% experienced a physician delay. There was a trend for patients with more severe loss of renal function to have a more direct referral to a nephrologist. Lack of or non-specific symptoms may contribute to substantially impaired renal function at diagnosis. Flu or upper-respiratory symptoms delayed diagnosis and referral to a nephrologist. Disease awareness and algorithms for faster diagnosis of renal involvement are needed for general and specialty physicians.

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Malignancy in MPO-ANCA Associated Vasculitis

Y. Arimura, M. Karube, T. Yamato, K. Nakabayashi,
A. Yamada, T. Nagasawa, Y. Takashima

Kyorin University School of Medicine, Internal Medicine,
Mitaka-city, Tokyo, Japan

Objective: The relationship between malignancy and myeloperoxidase(MPO)-antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis was investigated.

Methods: A retrospective review of 89 consecutive patients with MPO-ANCA associated vasculitis admitted to Kyorin University Hospital and affiliated hospitals was performed. The average age was 64.6 years old and male to female ratio was 1 to 1.4. The incidence of malignancy was compared with a normal population in Japan. Also, the previous reported cases of ANCA associated vasculitis complicated with malignancy in Japan were reviewed.

Results: Six patients in 89 MPO-ANCA associated vasculitis (6.7%) had a diagnosis of malignancy, 3 had lung cancer, 1 had prostate cancer, 1 had colon cancer and 1 had acute myeloid leukemia. The average age of the patients with malignancy was 68.3 years old, male to female ratio was 2 to 1. The rate of malignancy compared with an age-matched control group was increased in patients with MPO-ANCA-associated vasculitis. From 60 to 79 years old, standardized incidence rate ratio of malignancy for males was 5.2 and for females was 1.7. Twenty one ANCA associated vasculitis patients with malignancy were reported in Japan. Nineteen had MPO-ANCA or P-ANCA, 3 had PR3-ANCA or C-ANCA and 1 had both P-ANCA and C-ANCA. The average age was 65.3 years old, male to female ratio was 6 to 1. Seven patients (33%) had lung cancer, 6 (29%) had malignancy of the digestive tract, and 5 (24%) had hematologic malignancy (2 had acute myeloid leukemia, 3 had malignant lymphoma).

Conclusion: Patients with MPO-ANCA-associated vasculitis have an increased risk of malignancy. Malignancy in MPO-ANCA-associated vasculitis was dominantly male. Main malignancy was lung cancer.

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European Medicines Agency (EMA) Consensus on the Classification of the Primary Systemic Vasculitides (PSV) for Epidemiological Studies

S.E. Lane, R.A. Watts, D.G.I. Scott on behalf of
EMA vasculitis group

Ipswich Hospital, Rheumatology, Ipswich, UK

Background: There is no ideal classification scheme for PSV [Wegener's Granulomatosis (WG), microscopic polyangiitis (MPA), Churg Strauss syndrome (CSS), polyarteritis nodosa (PAN)]. Application of both ACR and Chapel Hill Consensus Conference definitions (CHCC) to the same patient in parallel often produces conflicting classification. MPA only features in CHCC definitions. ANCA are not included in either. A group of physicians studying the epidemiology of vasculitis met at the EMA. The aim was to develop a systematic scheme using ACR and CHCC criteria to classify individual cases into a single diagnosis.

Methods: An algorithm was devised applying the ACR and CHCC criteria stepwise. The ACR criteria were used first because they have been validated and CHCC considered MPA a diagnosis of exclusion. ACR criteria for PAN was not used due to poor specificity. Surrogate markers for WG were symptoms of granulomatous disease either i) pulmonary infiltrates/cavitations for >1 month on X-ray or ii) bloody nasal discharge/crusting for >1 month, chronic sinusitis, otitis media, mastoiditis, acute hearing loss, retroorbital disease, subglottic stenosis or saddle nose. Markers for renal vasculitis were proteinuria/haematuria with red cell casts. Each participating centre applied the algorithm to a cohort of their patients and the algorithm modified appropriately. We applied the new algorithm to a cohort of 99 patients to test whether it produced only a single category. The outcome was compared with the clinical diagnosis and classification using ACR criteria (WG, CSS PAN), CHCC definitions (WG, CSS, MPA, classic PAN-cPAN) and Lanham criteria (CSS).

Results: The figure shows the algorithm and table 1 the results of applying classification criteria in parallel to 99 patients.

*Clinical diagnosis must comply with a) At least 1 of i. histological vasculitis/granuloma, ii. positive ANCA iii. specific investigations strongly suggestive of vasculitis/granuloma AND/OR b) symptoms characteristic [if a) false] or compatible [if a) true] with diagnosis of PSV AND c) No other diagnosis to account for symptoms and signs

**Fulfills either ACR or Lanham classification criteria for CSS

***Either a) Histology compatible with CHCC WG, b) Histology compatible with CHCC MPA and surrogate markers for granulomatous inflammation (WG-markers) or c) No histology but WG-markers and positive ANCA (either cANCA/PR3 or pANCA/MPA)

#MPA: Either a) Clinical features and histology compatible with CHCC MPA, No WG-markers or b) No histology but surrogate markers for renal vasculitis and cANCA/PR3 or pANCA/MPA present

##Histology or angiographic features compatible with CHCC PAN

Table 1 for Abstract 110. Comparison of Classification Systems

	WG	WG and CSS	CSS	PAN	MPA	cPAN	Unclassified
ACR	54	1	15	60*	n/a	0	11
CHCC**	12	0	4	n/a	46	0	37
Lanham	n/a	n/a	16	n/a	n/a	n/a	83
EMEA	56	n/a	16	n/a	24	0	3

n/a 5 not applicable.

Conclusion: The algorithm appears to provide a mostly inclusive method of classification for PSV. It is being tested further using paper cases by each participating centre.

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Standardized Patient Education is Effective in Systemic Vasculitis

K. Herlyn, W.L. Gross, J. Hoeder, E. Reinhold-Keller

UK-SH, Rheumatology, Lübeck, Germany

Background: Standardized patient education programs are effective as an additional therapy in chronic diseases and are able to reduce disease activity and depression. Most patient education programs base on cognitive behavioral interventions and are supposed to improve the patient's self-efficacy. A standardized interdisciplinary patient education program for primary systemic vasculitis (PSV) was developed and established in our department.

Purpose: To evaluate the therapeutic effect of the program with a prospective study in a pre/post design.

Methods: Our newly designed patient education program consists of 5 modules each conceived for 90 minutes interactive training based on information presented on transparencies by physicians, psychologists, nurses, dieticians and physiotherapists. To evaluate the program and measure the therapeutic effect a documentation system with physician- and patient-administered questionnaires assessing different aspects of health status as health-related quality of life (SF-36), disease extent (DEI) and -activity (BVAS), laboratory parameters (ESR, CRP, ANCA), employment status, disability, knowledge (medicine, physiotherapy, nutrition) and self efficacy was developed and completed before, 1, 6 and 12 months after participating in the program.

Results: 69 patients (30% male, 70% female, mean age 55.2 years (range 23–87)) participated in 7 closed groups. 50% were diagnosed with ANCA-associated PSV. Knowledge improved statistically significant in medicine ($p < 0.001$), physiotherapy ($p < 0.05$) and nutrition ($p < 0.01$) between T0 and T1/T2. All components of health-related quality of life improved, with statistically significant higher estimates in pain, social function, mental health and physical role. Patients older than 60 years had a sign. lower knowledge at baseline than younger patients, but improved statistically significantly at T1 and T2.

Conclusions: We present the first standardized patient education program for PSV. Knowledge and HRQL improved significantly over a period of 12 months. The practicability and acceptance of the program were high. These results indicate an effect of the program.

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Stable Incidence Rates of Primary Systemic Vasculitides over Seven Years: Results of the German Vasculitis Register

K. Herlyn, R. Wagner-Bastmeyer, W.L. Gross, E. Reinhold-Keller

UK-SH, Rheumatology, Lübeck, Germany

Objective: To register all newly diagnosed patients with primary systemic vasculitides (PSV) in a large region in North Germany. To estimate the yearly incidence rates of PSV and their stability in a large population-based prospective study.

Methods: From 01/01/1998 until 31/12/2004 all new cases of PSV as defined by the Chapel Hill Consensus Conference 1992 were identified in a large mixed rural/urban region in Northern Germany consisting of 2,823,171 habitants. Information was obtained from (a) all departments of all hospitals, including their outpatient clinics; (b) all departments of pathology, and (c) the reference immunological laboratories serving the catchment area.

Results: Within the period of seven years 855 patients were diagnosed with PSV. The incidence rates for all PSV vary between 40 and 54 cases per one Million/Year. The most frequent PSV was Giant cell arteritis with incidence rates between 25 and 46 in the population older than 50 years. Incidence rates of ANCA associated PSV (Wegeners granulomatosis (WG), microscopic polyangiitis (MPA), Churg Strauss syndrome (CSS)) were between 9.5 and 16/million/year. WG occurred two to three times more frequently than MPA and CSS. The results are consistent over the period of seven years.

Conclusion: Results of a population based vasculitis register over 7 years for the incidence of PSV serving for 2.8 million habitants in north Germany revealed a stable incidence for all PSV. Compared to other European studies from small regions or referral centers the incidence rates for ANCA associated PSV, e.g. WG in this study (6–12) were comparable to Norway (8), lower than those in United Kingdom (10.6), but higher than in Spain (2.95).

Treatment of Refractory Henoch-Schönlein Purpura with Intravenous Immunoglobulin

C.P. García, M. Etcheverría, R. Labeaga, J. Ibáñez,
J. Sánchez, M. Arteaga, R. Campos

Department of Internal Medicine, Virgen del Camino
Hospital, Internal Medicine, Pamplona (Navarra), Spain

Background: Henoch-Schönlein purpura (HSP) is a systemic vasculitis, with IgA-dominant immune deposits, affecting small vessels. Typically involves skin, gut, glomeruli, and joints. There is no specific treatment for HSP. Glucocorticosteroid therapy does not appear to lessen the chances of recurrences. Furthermore, the evidence to date does not show that immunosuppressive therapy alters the natural history of disease.

Objective: To report, the efficacy of intravenous immunoglobulin (IVIG) in two patients with refractory HSP.

Patients and Methods: Patient 1. A 42-years-old man was admitted with fever, malaise, headache, irritability, arthralgia, abdominal pain, and a nonpruritic purpuric rash. Over the preceding 6 years he had experienced all these features during nine similar episodes. A skin biopsy specimen leukocytoclastic vasculitis. Immunofluorescence studies showed IgA and C3 along the small vessels of the skin. Because of the relapsing course of the HSP in this patient and the patient's lack of response to corticosteroids, the patient was treated with IVIG (total dose, 2 g/kg). Rapid resolution of his symptoms occurred, and the patient remains without relapse 36 months after therapy. Patient 2. A 17-year-old man had a 6-year history of recurrent episodes of fever, arthralgia, abdominal pain, tarry stools, and a purpuric rash on the lower limbs. A skin biopsy specimen showed leukocytoclastic vasculitis. Immunofluorescence studies showed with deposition of IgA, C3, and fibrinogen. After the absence of response to standard therapy, he was treated with IVIG (total dose, 2 g/kg) with prolonged remission of the symptoms.

Conclusion: IVIG may be useful in the treatment of patients with refractory HSP.

Characteristics of Giant Cell Arteritis in Latin American Patients

L.F. Flores-Suarez, J. Mena

Instituto Nacional de Nutrición, Immunology and
Rheumatology, D.F., Mexico

Objective: Description of clinical, laboratory and histopathological features of a population of Latin American patients with giant cell arteritis (GCA). Material and Methods: Retrospective data review (1989–2004). Comparisons are made with cohorts in Europe and USA.

Results: 14 patients with GCA were identified. Two were excluded due to insufficient data. Of the remaining 12 patients, 10 were women and 2 men. All were Mexican mestizos, except one whose father was Italian. Diagnosis was made at a mean of 16.5 ± 17.5 weeks from symptoms onset. Mean age at diagnosis: 73 ± 9 years. Most frequent presenting symptoms: headache (41%), fever and amaurosis fugax (25%); only one patient had polymyalgia rheumatica (PMR) as onset syndrome. In the course of the disease headache (83%), malaise and fever (58%) were the most frequent symptoms, followed by scalp tenderness, visual loss and PMR (50%): Upper limb claudication and fever of unknown origin were the less frequent. High erythrocyte sedimentation rate (ESR) was present in 83%, mean haemoglobin: 12.5 ± 1.8 g/dL, platelets $411,400 \pm 207,500/\mu\text{L}$, and in all 3 cases where C-reactive protein was measured, the value was high. In 9 cases, temporal artery biopsy was done. Four had characteristic findings, one had mild inflammatory infiltrate, one was inadequate for diagnosis and 3 were normal. No patient had bilateral biopsy. ACR criteria were fulfilled in all (at least 3; five patients had 4 criteria). Response to prednisone (mean dose 50 mg qd) was observed in all with improvement in a mean of 2.5 weeks. Three patients received IV methylprednisolone due to amaurosis fugax. Two patients had relapse possibly related to rapid steroid tapering. Additional treatment was methotrexate, azathioprine and cyclophosphamide ($n = 2$ for each). Median follow-up: 24 months (1–120).

Discussion: Although small, our series (the first known to be reported from a Latin American country in a Mestizo population), shows a higher frequency of fever and amaurosis fugax and less frequent PMR at onset in comparison to others, as well as during the course of the disease. High ESR was less and leukocytosis more frequent when compared with other series. Bilateral temporal biopsy was never done. This needs modification, especially as we had a high frequency of normal or unspecific results with no complications of the procedure. A good response to steroid treatment was observed.

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