2026

DEVELOPMENT OF CUTANEOUS VASCULITIS INDUCED BY IMMUNE COMPLEX DEPOSITION REQUIRES EXPRESSION OF ICAM-1 AND L-SELECTIN. Yuko Kaburagi, Shinichi Sato, Douglas A Steeber, Thomas F Tedder, Kazuhiko Takehara Kanazawa, Ishikawa, Japan and Durham, NC

The perivascular deposition of immune complexes induces acute inflammatory response with tissue injury. The immune-complex injury has been implicated in various human diseases, especially vasculitis. Cutaneous vasculitis induced by immune complexes is mediated by inflammatory cell infiltration that is highly regulated by expression of various adhesion molecules. However, the role of adhesion molecules, including L-selectin and intercellular adhesion molecule-1 (ICAM-1), is not known in this pathogenetic process. We examined the cutaneous reverse passive Arthus reaction, which is a classic experimental model for immune complex-mediated vasculitis, in mice lacking L-selectin, ICAM-1, or both. The reverse Arthus reaction was induced by intradermal injection of anti-chicken egg albumin antibody, followed immediately by intravenous injection of chicken egg albumin. Edema formation at 4 hours after injection was reduced in ICAM-1-/- (43% decrease), L-selectin-/- (31%), and L-selectin/ICAM-1-/- mice (51%) compared with wild-type mice ($p<0.0001$). The loss of both ICAM-1 and L-selectin resulted in a significant reduction of extravasation relative to L-seletin loss alone, but not to ICAM-1 loss alone. Simiarly, hemorrhage at 8 hours was inhibited in ICAM-1−/− (48% decrease), L-selectin loss alone, but not to ICAM-1 loss alone. Simiarly, hemorrhage at 8 hours was inhibited in ICAM-1−/− (48% decrease), L-selectin−/− (45%), and L-selectin/ICAM-1−/− mice (64%) compared with wild-type mice ($p<0.005$). L-selectin/ICAM-1−/− mice exhibited significantly reduced hemorrhage compared with ICAM-1−/− and L-selectin−/− mice. The edema formation at 4 hours was more strongly inhibited in ICAM-1−/− mice than L-selectin−/− mice while this difference was not detected for hemorrhage at 8 hours, suggesting that ICAM-1 functions earlier than L-selectin. The inhibited edema and hemorrhage were associated with reduced infiltration of neutrophils in each deficient mouse. Mast cell infiltration was dramatically reduced after 4 hours in each deficient mice relative to wild-type mice ($p<0.0001$). Furthermore, the production of tumor necrosis factor-α that was generated by neutrophils and mast cells was inhibited in each deficient mouse. These results indicate that ICAM-1 and L-selectin cooperatively contribute to cutaneous vasculitis by regulating the infiltration of neutrophils and mast cells. These results also suggest that ICAM-1 and L-selectin would be therapeutic targets as anti-adhesion therapy for vasculitis.

Disclosure:

2027

MEFV MUTATIONS ARE INCREASED IN BEHCET S DISEASE (BD) AND ASSOCIATE WITH VASCULAR INVOLVEMENT. Pamir Atagunduz, Tulin Ergun, Haner Direskeneli Istanbul, Turkey

Introduction: Mutations of the MEFV gene, which encodes the neutrophil protein pyrin (marenostrin) was recently linked to familial Mediterranean fever (FMF), an autosomal recessive, periodic inflammatory disease. The association of MEFV mutations with BD, a systemic vasculitic disorder suggested to have a neutrophil hyperfunction, was also reported. The role of MEFV mutations in the pathogenesis of BD was further investigated in a population with high frequencies of both disorders.

Methods: We screened a cohort of 50 BD patients for M694V, M680I and V726A mutations with ARMS method using PCR. All patients fulfilled the international criteria for BD and have been screened for a possible diagnosis of FMF with revised Tel-Hashomer criteria. The results were compared to a non-inflammatory control group (n=186).

Results: MEFV mutations were found in 24% (12/50) of BD patients compared to 9.1% (17/186) of the controls ($p=0.008$)(M694V in 9, V726A in 3 and M680I in one patient). All patients were heterozygous for MEFV mutations except for one patient homozygous for M694V. Out of 12 BD patients with MEFV mutations, 8 had vascular involvement compared to 5 patients in the mutation-negative group ($p=0.0007$). Four out of 13 patients with vascular involvement had severe vascular complications such as Budd-Chiari syndrome, vena-cava superior thrombosis and vascular neuro-BD, all in mutation-positive group ($p=0.002$). Patergy test was also more frequently positive in the MEFV mutation group (10/12 vs 16/35, $p=0.04$), whereas the frequency of uveitis did not differ in both groups (4/12 vs 8/38, $p=0.45$).

Conclusion: MEFV mutations, especially M694V, may act as genetic susceptibility factors in BD. The association of only vascular manifestations, but not uveitis with MEFV mutations also suggests that neutrophil-related genetic predispositions might be linked to certain clinical subsets of BD.

Disclosure:

2028

CHLAMYDIA PNEUMONIAE AND TEMPORAL ARTERITIS: NO ASSOCIATION BY POLYMERASE CHAIN REACTION (PCR) ANALYSES OF TEMPORAL ARTERY BIOPSIES. M J Regan, B J Wood, Y-H Hsieh, M L Theodore, T C Quinn, D B Hellmann, W R Green, C A Gaydos, J H Stone Baltimore, MD

PURPOSE: To examine the reported correlation between the presence of *Chlamydia pneumoniae* in temporal artery biopsy specimens and the diagnosis of temporal arteritis (TA).

METHODS: We reviewed reports of all the temporal artery biopsies performed at our institution between 1968 and 2000, identifying 90 possible cases of TA. Seventy-nine of the biopsy specimens (88%) demonstrated giant cells; 11 cases (12%) had other histopathological features compatible with TA. Through a rigorous chart review, we confirmed that all 90 patients with positive biopsies met the 1990 ACR criteria for TA. We chose controls from the group of individuals who had negative temporal artery biopsies during the same time 32-year period. We reviewed the charts of potential controls to ensure that their post-biopsy courses were not compatible with TA, and matched one control to each case on 3 variables: gender, year of biopsy, and age within 10 years. The biopsies of all cases and controls were re-evaluated in a masked fashion by an experienced eye pathologist; all of the original readings were confirmed. Following de-paraffinization of the samples and DNA extraction, PCR analyses were performed for *C. pneumoniae* on the 180 samples. We used 2 CDC-recommended sets of PCR primers (targeting 2 different genes) for *C. pneumoniae*. A primer set targeting the *ompA* gene (CP1-CP2/CPC-CPD) was used to perform a nested PCR, followed by confirmation of the findings with primers targeting the 16S rRNA gene in a touchdown enzyme, time-released PCR (CPN90/CPN91). We used positive and negative controls as well as controls made from infected and non-infected Hep-2 cells, suspended in a formalin-fixed, paraffin-embedded matrix.

RESULTS: The results of PCR analyses are shown below.

PCR primer set	CASES (Positive TA bx)	CONTROLS (Negative TA bx)
	N=90	N=90
ompA	1 (1.1%)	1 (1.1%)
16SrRNA gene	0	0

CONCLUSIONS: The results of this comprehensive study, which involved a large number of biopsy-proven cases of TA and matched controls and employed sensitive and specific PCR analyses, do not support an association of *C. pneumoniae* in the pathogenesis of TA.

Disclosure: Supported by a grant from the NIH (Grant number 1R21HL65099-01) and the Peggy Meyerhoff Pearlstone Foundation.

2029

CHEMOKINE RECEPTOR EXPRESSION ON CD4-POSITIVE CD45RO-POSITIVE MEMORY T-CELLS IN WEGENER'S GRANULOMATOSIS. Peter Lamprecht, Anika Erdmann, Antje Mueller, Elena Csernok, Wolfgang L Gross Luebeck, Germany

Objective: Chemokine receptors play an important role in lymphocyte activation and recruitment to sites of inflammation. In Wegener's granulomatosis (WG) granulomatous lesions contain abundant CD4-positive T-cells. We analyzed, whether peripheral blood CD4-positive CD45RO-positive T-cells express chemokine receptors suggestive of their capability to respond to chemotactic gradients and of coordinated T-cell migration.

Methods: Patients with biopsy-proven localized WG (n=5), generalized WG (n=17) and age-and sex-matched healthy controls (HC, n=13) were included. PBMC were isolated and labeled with fluorochrome-conjugated monoclonal antibodies for cell surface antigens or appropriate negative (isotype) controls. Expression of CCR3, CCR5 and CXCR3 was determined by flow cytometry (FACS). Lymphocytes were gated for analysis based on light scattering properties and on CD45 and CD4 staining. Positively and negatively stained populations were calculated by quadrant dot plot analysis determined by isotype controls.

Results: The fractions of CCR5-positive and CCR3-positive cells within the CD4-positive CD45RO-positive T-cell population were significantly expanded in localized WG (mean: 15% CCR5-positive T-cells resp. 7% CCR3-positive T-cells) and generalized WG (4% resp. 4%) as compared with HC (1.5%resp. 1.2%, $P<0.01$). CCR5/CCR3 coespression was not different in WG or HC (10%). Simultaneous expression of CCR5 and CXCR3 was detected on 24% resp. 11% of CD4-positive CD45RO-positive in localized resp. generalized WG compared with 6% in HC ($P<0.05$). CCR5 expression on CD28-negative CD4-positive T-cells was higher in localized WG as compared with generalized WG (66% vs. 12%, $P<0.05$).2

Conclusion: Upregulated CCR5 or CCR3 expression on CD4-positive CD45RO-positive memory T-cells indicates previous activation and homing capability of different memory T-cell subsets in WG. Moreover, CD28-negative T-cells displayed CCR5 expression consistent with their Th1-like cytokine production, which has been demonstrated by our group recently.

Disclosure: Supported by grant SFB367/A8 to PL, AM, and WLG.

2030

INFLUENCE OF CCR5, CCR5Δ32, RANTES AND MIP CHEMOKINES IN WEGENER'S GRANULOMATOSIS (WG). Yihua Zhou, DeRen Huang, Carol Farver, Gary S Hoffman Cleveland Ohio

Background: During inflammation, chemokines control emigration of leukocytes via their G-protein-coupled cell surface receptors. CC chemokine receptor 5 (CCR5) is the receptor for the β-chemokines including RANTES, MIP-1α and MIP-1β. CCR5 is expressed mainly on macrophages, Th1 T cells and dendritic cells. CCR5 Δ32, a naturally occurring variant of CCR5, has a 32 bp deletion (Δ32) that results in a non-functional receptor. Leukocytes from individuals heterozygous for CCR5 Δ32 express significantly lower levels of CCR5.

Patients and Methods: This study investigated allelic and genotypic frequencies of polymorphisms located in genes encoding these chemokines and receptors in 118 Caucasian patients with WG and 127 ethnically matched healthy controls. In addition, protein expression of CCR5 and ligands was studied using immunohistochemistry staining in 4 lung biopsies that had classical features of WG.

Results: Enhanced protein levels of RANTES, MIP-1α and MIP-1β were noted in lung lesions from patients with WG, which was mirrored by the elevated levels of CCR5 expression. These results suggest a critical role for CCR5 signaling in local inflammatory responses of WG.

Genetic analyses revealed similar allelic frequencies and genotypic distributions of CCR5 Δ32 in patients and controls. However, among patients in whom circulating ANCA was not detected, none were found to carry the CCR5 Δ32 allele. The significant under-presentation of CCR5 Δ32 in patients without ANCA (0/19, 16% of WG cohort) suggests that CCR5 signaling exerts an essential role and maximal impact in WG patients with the minimal influence of ANCA.

Polymorphisms located in the promoter regions of the gene encoding RANTES were also examined. No significant difference was noted between patients, subsets of patients and controls, suggesting that genetic polymorphisms in RANTES do not influence expression of WG.

Conclusions: Our data suggest an important role for CCR5 in the mononuclear inflammatory lesions of WG. Strategies to block CCR5 ligation may be useful in the treatment of WG.

Disclosure:

2031

CD4-POSITIVE CD28-NEGATIVE T-CELLS ARE THE MAJOR SOURCE OF TH-1 LIKE CYTOKINE PRODUCTION IN WEGENERS'S GRANULOMATOSIS. Peter Lamprecht, Andras Komocsi, Elena Csemok, Antje Mueller, Ulrike Seitzer, Frank Moosig, Armin Schnabel, Wolfgang L Gross Luebeck and Borstel, Germany

Objective: Expansion of T-cells lacking CD28 expression has been reported in Wegener's granulomatosis (WG) previously. We studied, whether the fraction of CD28-negative T-cells within the CD4-positive T-cell population is a major source of cytokine production.

Methods: 12 patients with active WG were analyzed. We assessed surface antigens and intracytoplasmic cytokine expression of the peripheral blood fractions of CD28-negative and CD28-positive T-cells within the CD4-positive T-cell population by flow-cytometry (FACS). Cytokine secretion was additionally confirmed by an enzyme-linked immunosorbent assay (ELISA). Immunohistologic studies were performed on biopsies from the respiratory tract.

Results: The fraction of CD28-negative T-cells within the CD4-positive T-cell population was significantly expanded compared with healthy controls (mean 14.4 vs. 2.1%, $P<0.01$). CD4-positive CD28-negative T-cells expressed CD18, CD57 - a marker also found on NK-cells - and intracytoplasmic perforin. They generally lacked the activation marker CD25 (IL-2receptor/IL-2R). CD4-positive CD28-negative T-cells appeared as the major source of IFN-γ- and TNF-α production and secretion. In contrast, CD4-positive CD28-positive T-cells expressed CD25, but no perforin, and were able to produce and secrete a wider variety of cytokines, including IL-2. Immunohistologic analysis demonstrated that the majority of T-cells lacked CD28 in granulomatous lesions. They generally lacked the activation marker CD25 (IL-2receptor/IL-2R). CD4-positive CD28-negative T-cells appeared as the major source of IFN-g- and TNF-a production and secretion. In contrast, CD4-positive CD28-positive T-cells expressed CD25, but no perforin, and were able to produce and secrete a wider variety of cytokines, including IL-2. Immunohistologic analysis demonstrated that the majority of T-cells lacked CD28 in granulomatous lesions.

Conclusion: CD4-positive CD28-negative T-cells appeared highly differentiated, displayed a Th1-like cytokine production and features suggestive of T-cell mediated cytotoxicity. β2 integrins, i.e. CD18, may promote recruitment of CD4-positive CD28-negative T-cells into granulomatous lesions, where they may promote granuloma formation by IFN-γ- and TNF-α secretion.

Disclosure: Supported by grants SFB367/A8 to PL, AM, and WLG, SFB367/C1 to US, and a German Academic Exchange Service grant to AK.

2032

BLYS MEDIATES HOMEOSTATIC SURVIVAL IN IMMATURE AND MATURE B CELLS VIA DISTINCT MECHANISMS. Benjamin L Hsu, David M Hilbert, Michael P Cancro Philadelphia, PA and Rockville, MD

B Lymphocyte Stimulator (BLyS), a recently described TNF superfamily member, acts specifically upon B lymphocytes and is elevated in serum of patients with rheumatoid arthritis or systemic lupus erythematosus. BLyS is an important regulator of B cell homeostasis, since administration of exogenous BLyS to mice causes a marked increase in peripheral B cell numbers, whereas BLyS antagonists reduce B cell numbers. Which B cell subsets expand in response to BLyS, and how BLyS increases B cell numbers, are still unclear. Because these issues potentially affect the composition of the B cell pool and antibody repertoire, we examined the binding and activity of BLyS on defined subsets of immature and mature murine B cells.

First, we demonstrated by FACS analysis that soluble recombinant BLyS (rBLyS) binding is limited to surface Ig+ B cells in the bone marrow (BM) and spleen. Bone marrow pro-B and pre-B cells did not bind BLyS. Additionally, rBLyS binding to mature B cells was greater than to immature B cells of the BM or spleen. At the mRNA level, the BLyS receptor genes, TACI and BCMA, were differentially expressed during B cell maturation from immature BM cells to resting, mature splenic B cells. TACI transcripts were more abundant in mature peripheral B cells.

Secondly, we examined the functional effect of culturing primary mature or immature splenic B cells with rBLyS, and analyzed expression of the gene *A1/Bfl-1*. This anti-apoptotic member of the Bcl-2 family is upregulated by the NFkB pathway. BLyS signaling through BCMA and TACI activates the NFkB pathway also, and thus its biological effects may be mediated through survival-promoting genes such as *A1/Bfl-1*. Results of a semiquantitative RT-PCR assay for *A1/Bfl-1* mRNA showed that for mature splenic B cells, rBLyS induces *A1/Bfl-1* expression in this subset. In contrast, immature splenic B cells fail to upregulate *A1/ Bfl-1* in response to rBLyS. Despite this difference in anti-apoptotic gene response, both immature and mature B cells survive better in culture when BLyS is added. These results suggest that BLyS-mediated survival occurs via distinct mechanisms in B cell subsets. Shifts in proportionate survival of certain immature or mature cells may dynamically shape the B cell repertoire in normal or diseased states.

Disclosure:

2033

ROLE OF THE JAGGED / NOTCH DIFFERENTIATION PATHWAY IN IMMUNE REGULATION : POTENTIAL IMPLICATION IN RA. Frederique Ponchel, Wan Fai Ng, Jonathan Lamb, Robert Lechler, John Isaacs Leeds, Edinburgh and London, United Kingdom

Rheumatoid arthritis is a chronic inflammatory disease. Paradoxically, there is evidence for suppressed immune function in RA as well as specific T-cell defects, such as profound unresponsiveness, loss of proliferative capacity and IL-2 production. The cell fate decisions resulting in the development of either T-helper or T-regulatory cells depend upon signals received from APCs. Engagement of the TCR together with co-stimulatory signals and accessory adhesion molecules leads to full T-cell activation. The alterations of the signaling pathway that transform an activating signal into an anergic and/or regularoty signal are not clearly understood. One signaling pathway, which has the capacity to regulate cell fate in various tissues, is controlled by the *notch* receptor and its ligands, *jagged*. Recently, the *jagged/notch* pathway was shown to induce the differentiation of peripheral murine CD4 T-cells into regulatory cells, upon transfection of *jagged* on APCs. These regulatory cells could transfer tolerance to naive cells. Using models of human T-cell anergy and mouse tolerance, we quantified the expression of each member of the *notch/jagged* family and their down-stream effectors, during the induction of anergy and activation *in vitro* and *in vivo*. We observed down regulation of the *jagged* and *notch* genes throughout activation and immunisation. In contrast during anergy and tolerisation, expression of all genes initially rose and subsequently fell to base line. Furthermore, we established that there is induction of down stream signals from the *notch* receptor during anergy and tolerisation. Human regulatory cells have recently been described as CD4+/CD25+ T-cells. We have analyzed the expression of *jagged/notch* and downstream signaling molecules in sorted regulatory cells and compared it to CD4+/CD25- cells. We observed minor differences between both populations. However, when CD24+/CD25+ cells were activated, the expression of the different components of the pathway as well as the down stream signals was modulated. These data suggest an important role for the *notch/jagged* family in the regulation of anergy, tolerance and during a regulatory response. We subsequently studied expression of *jagged/notch* family in peripheral blood mononuclear cells from newly diagnosed RA patients and also compared blood and synovial fluid mononuclear cells from resistant disease patients. Our data suggest a difference in the pattern of expression of these genes between RA and healthy controls and further differences were observed between blood and synovial fluid cells. These pilot data suggest a potential role for the *jagged/notch* differentiation pathway in RA, which may relate to the immunological defects chatacteristic of RA.

Disclosure:

2034

DEREGULATED Ras AND Rap1 SIGNALING IN RHEUMATOID ARTHRITIS SYNOVIAL FLUID T LYMPHOCYTES LEADS TO PERSISTENT REACTIVE OXYGEN SPECIES PRODUCTION AND CHRONIC OXIDATIVE STRESS. Philip HJ Remans, Jaap M van Laar, Kris A Reedquist, Ellen AM Papendrecht, Nivine EW Levarht, Johannes L Bos, Ferdinand C Breedveld, Cornelis L Verweij, Sonja I Gringhuis Leiden and Utrecht, The Netherlands

In rheumatoid arthritis (RA), the synovial fluid (SF) T lymphocytes present in the inflamed joints display hyporesponsiveness upon engagement of the TCR/CD3 complex despite phenotypic evidence of former activation. Previously, we have provided evidence that the hyporesponsiveness of the SF T lymphocytes is due to the membrane displacement of the signaling protein LAT (linker for activation of T cells), which plays a central role in the T cell receptor (TCR)-mediated signaling pathways, as a result of severely decreased intracellular levels of the antioxidant glutathione (GSH), a hallmark of chronic oxidative stress. In this study, we set out to elucidate the source of the chronic oxidative stress.

Using pull-down assays to determine the activation status of the small GTPases Ras and Rap1, we observed that Ras is constitutively active in SF T lymphocytes, while Rap1 remains in the inactive GDP-bound state even after stimulation of the cells. In contrast, both Ras and Rap1 are inactive in resting peripheral blood (PB) T lymphocytes, where they become active after stimulation through the TCR/CD3 complex (Ras and Rap1) or with H_2O_2 (Rap1).

Using transient transfections experiments in combination with a FACS assay to determine the presence of reactive oxygen species (ROS) by measuring the fluorescence of oxidized DCF, we established that active Ras is sufficient for the continuous generation of ROS. Furthermore, we found that active Rap1 through a phosphatidylinositol 3-kinase (PI-3K)-dependent pathway inhibits the Ras-dependent production of ROS. Transfected cells expressing either an inactive GDP-bound Rap1 mutant (Rap1- N17), a Rap1 mutant lacking its membrane-binding domain (Rap1D CAAX), or a deactivator of Rap1 (RapGAP) showed enhanced ROS generation after stimulation compared with normal cells. These data indicate that in normal cells the generation of ROS through activation of Ras is downregulated by Rap1.

We conclude that SF T lymphocytes from RA patients have a defective mechanism for the downregulation of ROS production, which leads to intracellular chronic oxidative stress and consequently hyporesponsiveness of the cells.

Disclosure:

2035

SLAP-130/FYB IS REQUIRED FOR TCR-DEPENDENT INTEGRIN ACTIVATION AND PROLIFERATION. Erik J Peterson, Melody L Woods, Sally A Dmowski, Geo Derimanov, Martha S Jordan, Jennifer N Wu, Peggy S Myung, Qing-Hua Liu, Jonathan T Pribila, Bruce D Freedman, Yoji Shimizu, Gary A Koretzky Philadelphia, PA; Minneapolis, MN

The hematopoeitic-specific adapter SH2-containing Leukocyte Protein of 76 Kda (SLP-76) plays critical roles in thymocyte development and T cell receptor (TCR)-dependent signal transduction. SLP-76 Associated Phosphoprotein of 130 kdA(SLAP-130, also known as fyb) is an adapter which associates with SLP-76 following TCR engagement. To investigate the biological role of SLAP-130/Fyb, we generated SLAP-130/fyb-deficient mice. SLAP-130/fyb-/- animals are viable, and in contrast to SLP-76-mutant mice, exhibit no bleeding diathesis. Unlike SLP-76 mutant mice, SLAP-130/fyb-/- animals produce mature T cells that populate spleen and lymph nodes. Furthermore, SLAP-130/fyb-/- T cells do not resemble a SLP-76-deficient T cell line, which displays absent biochemical responses after TCR engagement. Rather, purified T cells from SLAP-130/Fyb-/- lymph node or spleen exhibit TCR-dependent phosphorylation of protein tyrosine kinase substrates, calcium elevation, and MAPK activation comparable to control. Despite normal measures of TCR-proximal signaling, SLAP-130/Fyb-/- T cells show profound deficits in proliferation and IL-2 production in response to anti-CD3 stimuli. SLAP-130/Fyb has recently been implicated in integrin function. We therefore examined the ability of SLAP-130/Fyb-/- T cells to adhere to ligands for b1 and b2 family integrins. TCR ligation normally induces cellular adhesion to ICAM-1 or VCAM-1. SLAP-130/Fyb-/- T cells show complete absence of TCR-augmented adhesion to these molecules. Furthermore, TCR-induced lateral mobility of the Beta2 family integrin, LFA-1, is markedly defective in the mutant T cells. Together, these studies show that SLAP-130/Fyb is dispensable for key TCR-inducible signaling events, yet serves as a critical positive regulator of T cell activation, and is a required element in TCR-dependent "inside out" signaling to integrins.

Disclosure:

2036

FGF-1, A VASCULAR GROWTH FACTOR EXPRESSED IN RHEUMATOID SYNOVIUM, REGULATES T CELL RESPONSIVENESS TO CCR CHEMOKINES. Victor M Byrd, Geraldine G Miller, James W Thomas Nashville, TN

The chronic destructive lesion of rheumatoid arthritis is characterized by focal synovial injury with mesenchymal cell hyperplasia and neoangiogenesis. CD4+ T lymphocytes are thought to perpetuate these lesions, and are typically found clustered in perivascular aggregates. While the mechanisms that regulate T cell migration to these regions are unknown, small polypeptides known as chemokines likely contribute to lymphocyte trafficking from the vascular to the perivascular space. Such chemokine responsiveness may be determined by a complex interaction of cytokines and growth factors expressed at these sites. Specific vascular growth factors including fibroblast growth factor-1 (FGF-1) are known regulators of cellular proliferation in such lesions, but the mechanisms through which these factors regulate T cell migration are not known. Our previous work has shown that a subset of human CD4+ T cells express FGF receptors (FGFR1), and that FGFR1 activation results in enhanced DNA binding of NFAT and NF-κB transcription complexes, augmented IL-2 production, and T cell proliferation in vitro. In addition, these FGFR1+ T cells are increased in the chronic inflammatory lesions of RA and cardiac allograft rejection, suggesting that FGFs serve important functions in lymphoid as well as non-lymphoid responses. Recent investigations show that the FGFs have the capacity to regulate T migration to inflammatory sites through mechansims that modulate expression of specific CC chemokine receptors. Specifically, we find that FGF-1 stimulation in vitro upregulates human CD4+ T cell expression of several receptors for the CC chemokine family, including CCR-1, CCR-2a/b, CCR-4, and CCR-8. No effect on the CXC receptor family was found. The FGF response is most pronounced for CCR-4 expression, and occurs at 4-16 hours after FGF-stimulation. In addition, FGF-regulated CCR-4 RNA expression is not IL-2 dependent, and can not be attenuated by the addition of the protein synthesis inhibitor cycloheximde, suggesting that de novo synthesis of other cytokines and proteins is not required. Finally, we show that the CCR4-specific ligand macrophage-derived chemokine (MDC) is expressed by rheumatoid synovial fibroblasts, and that its expression is enhanced by FGF alone or in combination with other synovial growth factors such as TGF-β. Taken together, these results suggest novel mechanisms through which immune cells integrate signals generated through growth factor receptors to migrate and survey specific sites of tissue damage.

Disclosure:

2037

THE LUPUS SCOR COMBINED GENOME SCAN: IDENTIFICATION OF THREE MAJOR SUSCEPTIBILITY REGIONS. R P Kimberly, C D Langefeld, K L Moser, P M Gaffney, J Kelly, R R Graham, W M Brown, S S Rich, J B Harley, T W Behrens, for the SCOR in the Genetics of SLE Birmingham, AL; Winston-Salem, NC; Minneapolis, MN; Oklahoma City, OK

Twin studies and assessment of sibling risk of disease indicate that susceptibility for systemic lupus erythematosus (SLE) has a substantial genetic component. To accelerate the mapping of susceptibility regions and identification of genes predisposing to SLE and related autoimmune phenotypes, the Specialized Center of Research (SCOR) in the Genetics of SLE has performed a combined genome scan of 313 SLE pedigrees. These pedigrees, ascertained through 2 or more siblings with SLE, originate from collections assembled at the University of Minnesota (MN: 187 pedigrees, 656 individuals genotyped, 399 SLE patients) and the University of Oklahoma (OK: 126 pedigrees, 681 individuals genotyped, 298 SLE patients). Together the SCOR has constructed a combined map of 703 microsatellite markers (MN: 366, OK: 274, Both: 63) using marker order and distances available from the Mammalian Genotyping Center in Marshfield, WI. For initial analysis, we have computed multipoint nonparametric linkage regression analyses using the NPL (pairs) statistic and report the corresponding maximum LOD score (nearest marker, combined LOD, MN pedigrees LOD, OK pedigrees LOD). Three chromosomal regions approach or exceed genome-wide statistical significance with LOD>3.5:

chr 6 (D6S2410, LOD=4.90, MN LOD=4.30, OK LOD=1.03)
chr 4 (D4S403, LOD=3.65, MN LOD=1.79, OK LOD=1.91)
chr 16 (D16S3253/D16S503, LOD=3.51, MN LOD=4.86, OK LOD=0.50)

Additional regions of interest include: chr 1 (D1S2785, LOD=2.14); chr 4 (D4S2368, LOD=2.00); chr 7 (D7S507, LOD=2.47); and chr 20 (D20S481/D20S119, LOD=1.97). Interestingly, D1S2785 also represents the peak chr 1 results of Shai et al. on distal chr 1q (Human Molecular Genetics, 8: 639, 1999); and, with a parametric method, the Fc receptor cluster LOD exceeds 4.0 in OK. Future work includes testing for interactions among loci and investigating how the evidence for linkage varies by ethnic group and other phenotypic characteristics in order to focus the identification of predisposing genes.

Disclosure:

2038

RISK OF SLE IN RELATION TO ADMIXTURE IN WEST AFRICANS OVERSEAS. Mariam Molokhia, Alan L Patrick, Esteban J Parra, Jian Ye, Mark D Shriver, Alan J Silman, Paul M McKeigue London and Manchester, United Kingdom; Port of Spain, Trinidad and Tobago; State College, Pennsylvania

Objective: To distinguish between genetic and environmental explanations for high risk of SLE in west Africans compared with Europeans, by studying the relation of risk to individual admixture.

Methods: Cases of SLE, together with controls matched for age, sex and neighbourhood, were sampled from northern Trinidad, excluding those with Indian or Chinese names. Individuals were typed with a panel of 30 SNP markers chosen to have large frequency differentials between the three non-Asian parental populations (west African, European, Native American). Individual admixture (proportion of the genome that has ancestry from each parental population) was estimated from the marker genotypes.

Results: This analysis was restricted to the 53 cases and 102 controls who reported that they had no Indian or Chinese ancestry and had been genotyped. Mean proportion of African admixture (M) was estimated as 0.80 in cases, 0.74 in controls (p=0.03). In a logistic regression analysis, the risk ratio associated with unit change in M (from 0 to 1) was estimated as 17.6 (95% CI 1.3 - 239). This association was strengthened after adjustment in the analysis for socioeconomic factors and for religious affiliation.

Conclusion:

Risk of SLE in this admixed population is strongly related to the proportion of west African admixture, consistent with a genetic explanation for the higher risk of SLE in west Africans compared with other ethnic groups. This result provides a basis for designing studies to localize the genes underlying this effect by studying people of mixed descent using a panel of markers across the entire genome.

Disclosure:

2039

ASSOCIATION OF FCγR2A & 3A GENOTYPES WITH SLE: ANALYSIS OF 605 SLE FAMILIES. Lindsey A Criswell, Erin A Peden, Raymond F Lum, Victoria A Seligman, Jean L Olson, Hongzhe Li, Michael F Seldin San Francisco and Davis, CA

Objective: Several lines of evidence implicate a role for Fcγ receptor (FcγR) polymorphisms in SLE. Questions remain, however, about their relevance for patients with specific ethnic and clinical characteristics. Our goal was to define the roles of 2 functional polymorphisms using a large cohort of ethnically and clinically diverse SLE patients and family-based association methods.

Methods: We genotyped 605 SLE patients and 1,389 family members for the FcγR2A 131R/H and FcγR3A 158F/V polymorphisms. All patients met ACR criteria for SLE, and 148 patients were classified as lupus nephritis (LN) based on renal biopsies and/or fulfillment of ACR renal criteria. We used the family based association test (FBAT) to examine associations between each polymorphism and SLE, including the following subgroups: Caucasian, non-Caucasian, and LN.

Results: Examination of all 605 families revealed significant association of both polymorphisms with SLE susceptibility. Specifically, P values for the FBAT based on a dominant genetic model are: FcγR2A, p = 0.019; FcγR3A, p = 0.015. Ethnic subgroup analyses suggest that the FcγR2A polymorphism is more relevant for Caucasians (p = 0.0032) compared to non-Caucasians (p = 0.053). In contrast, the FcγR3A polymorphism is more strongly associated with SLE among non-Caucasians (p = 0.011) compared to Caucasians (p = 0.16). Comparison of results for different genetic models indicates that genotypes are more predictive of risk than individual alleles. Neither polymorphism reveals striking association with LN. However, power limitations preclude a definitive conclusion about the relevance of these polymorphisms for LN based on these data.

Conclusions: These results support an important role for FcγR polymorphism in SLE and document striking variation in risk depending upon ethnic background. Additional work is required to more precisely define the relevance of these polymorphisms for patients with specific clinical characteristics and for those with diverse ethnic backgrounds.

Disclosure:

2040

LINKAGE AND ASSOCIATION OF A FUNCTIONAL SINGLE NUCLEOTIDE POLYMORPHISM (SNP) IN THE FcγRIIIA GENE WITH SLE. J C Edberg, C D Langefeld, K L Moser, J Kelly, J M Kaufman, S S Rich, J B Harley, R P Kimberly, for the SCOR in the Genetics of SLE Birmingham, AL; Winston-Salem, NC; Minneapolis, MN; Oklahoma City, OK

Genome scan analyses of multiplex SLE families have identified multiple loci linked to the SLE diathesis. One locus, at 1q23, contains the low affinity Fcγ receptor cluster. Two candidate genes in this region, FcγRIIA and FcγRIIIA, contain functionally important SNPs in the coding region that alter the binding of IgG. Case-control studies provide evidence of an association between these SNPs and SLE. To test for family-based association not potentially confounded by population admixture, we genotyped and analyzed 126 multiplex families with SLE (61% Caucasians, 32% African-American); genotyping was done independently via two methods at two centers. Multipoint non-parametric linkage analysis of these SNPs provides evidence for linkage at both loci (FcγRIIA: NPL score=2.54 p<0.006; FcγRIIIA: NPL score=2.62, p<0.005). Analysis of family-based association using the transmission disequilibrium test (TDT) and the pedigree disequilibrium test (PDT) revealed strong association at the FcγRIIIA locus with little or no evidence of association at the FcγRIIA locus. These two SNPs are in significant linkage disequilibrium (p<0.0009), which may explain the potential trend for an association at FcγRIIA. Haplotype analysis did not provide strong evidence of an association with an extended haplotype (p=0.06).

Based on these results, the association between these SNPs and SLE is primarily due to the FcγRIIIA SNP where the risk allele was transmitted 61 times and not transmitted 28 times (TDT-based odds ratio=2.17, p=0.005). We have shown that this SNP modifies the binding of IgG1 and IgG3 leading to altered immune complex handling and altered inflammatory responses. This study implicates FcγR, and FcγRIIIA in particular, in susceptibility to SLE.

	Caucasions (n=77)		African-Americans (n=44)		All (n=126)	
	TDT	PDT	TDT	PDT	TDT	PDT
FcγRIIA	p=0.388	p=0.097	p=0.074	p=0.437	p=0.134	p=0.089
FcγRIIIA	p=0.012	p=0.044	p=0.016	p=0.020	p=0.0005	p=0.002

Disclosure:

2041

HEMOLYTIC ANEMIA IN PEDIGREES MULTIPLEX FOR LUPUS: IMPROVING POWER BY INCREASING GENETIC HOMOGENEITY. Gail R Bruner, Jennifer Kelly, Jeff Kilpatrick, Swapan K Nath, Bahram Namjou-Khales, R Hal Scofield, John B Harley Oklahoma City, Oklahoma

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder of unknown etiology. The hematologic abnormalities (hemolytic amenia, leukopenia, lymphopenia and thrombocytopenia) in SLE are classic manifestations of the disease. We evaluated hemolytic anemia as a selection criterion in an effort to identify a more homogeneous set of pedigrees multiplex for SLE and, hence, to reveal genetic efects not previously detected. From our collection of 160 pedigrees multiplex for SLE, we analyzed a sebset of 35 pedigrees (16 African-American(AA), 17 European-American(EA) and 2 Hispanic) that contained at least one SLE patient who also suffered from hemolytic anemia. Both non-parametric and maximum-likelihood model-based genome-wide linkage analyses were conducted using 328 microsatellite makers (Weber #8). In accordance with the recommendations for significant($LOD_{Est} \geq 3.3$, $p \leq 0.00002$) and suggestive linkage ($LOD_{Sug} \geq 1.9, p \leq 0.0017$, significant linkage to hemolytic anemia was identified at 11q14 in the AA pedigrees ($LOD_{max} = 5.22$), and at 1q24 (LOD=4.00) in the EA pedigrees. In addition, eight regions reached or surpassed the criterion for suggestive linkage. These are linkages important in lupus because they were identified in pedigrees multiplex for this phenotype. Their relationship to hemolytic anemia is, at present, unknown. Also, pedigree selection in SLE using this additional criterion appears to identify more genetically homogeneous pedigrees. Indeed, the 11q14 linkage in 16 AA pedigres is quantitatively much more impressive than any linkage identified to date using large collections of pedigrees with SLE alone as the phenotype. This example demonstrates that analyzing families selected by their clinical manifestations increases the power to dectect linkage for this complex disease and shows, again, the extraordinary power that clinical features have to inform disease genetics.

Disclosure:

2042

FORMATIVE RESEARCH ON PROMOTING PHYSICAL ACTIVITY AMONG PERSONS WITH ARTHRITIS. Teresa J Brady, Kathryn L Harben, Joseph E Sniezek Atlanta, GA

Selected physical activity is both safe and effective for people with arthritis (PWA), but PWA report significantly less leisure time physical activity than do the general population. This paper will summarize the formative research used to shape a health communications campaign designed to promote physical activity for PWA.

Formative research included a literature review, an environmental scan, a market research survey, physician interviews and 3 rounds of focus groups with PWA. Review of the literature demonstrated that the prevalence of arthritis increases after age 45, and that PWA with lower education and income levels experience more disability. Additional further formative research targeted this group: adults ages 45-64 with income below $35,000 and high school education or less.

Focus group findings indicated that most PWA had received non-specific recommendations to exercise from their physicians, but few were regularly performing moderate physical activity. The concept of physical activity was easily understood by participants. Pain relief and ability to do more or move more easily were reported as desired benefits, while time, pain, and inconvenience were key barriers. Common communications elements identified as motivating were lessening pain, increasing time and activities with family, and willingness to pay some cost now for some benefit later. Respondents indicated they were unlikely to seek information, but were interested in information they encountered.

Physician interviews confirmed that most primary care physicians recommend exercise; therefore, a physical activity campaign would be congruent with physician recommendations. The environmental scan revealed that most media coverage was directed at the promotion of medications, and that a number of other organizations were interested in reaching PWA and may be potential partners.

The formative research summarized here has shaped the development of health communications message content and delivery modes to promote physical activity to lower income and education level PWA. The campaign materials–radio and print ads and brochures ina countertop display—will be used to promote physical activity among people with arthritis.

Disclosure:

2043

EFFECTS OF 12-WEEK TAI CHI EXERCISE ON PAIN, BALANCE, MUSCLE STRENGTH, AND PHYSICAL FUNCTIONING IN OLDER PATIENTS WITH OSTEOARTHRITIS: RANDOMIZED TRIAL. Eun-Ok Lee, Rhayun Song, Sang-Cheol Bae Seoul and ChonAn, Republic of Korea

The Sun-style Tai Chi exercise has been developed specifically for arthritis patients in order to reduce their symptoms and to improve physical fitness and functioning. This randomized study examined the changes in pain, balance, muscle strength and physical functioning in older osteoarthritis patients at the completion of 12 week Tai Chi exercise.

The patients with osteoarthritis who signed the consent form were randomly assigned into two groups. 17 experimental subjects and 14 comparisons completed pretest and posttest measures at 12-week interval with 28% of dropout rate. Outcome measures were physical fitness and muscle strength (Takei Kiki Kogyo Co.& Cybex) and physical functioning (Korean-Womac). Data were entered and analyzed by SPSSWIN 10.0 program. Independence t-test was utilized to examine group differences.

The homogeneity test confirmed that there was no significant group difference in demographic data and pretest measures. The subjects were 64 years of age and have been diagnosed for 9.4 years in average. Most of them were still married (72%), and doing none(59%) or very seldom exercise(23%) previously. 30.2% of the subjects quit the job due to their illness.

At the completion of 12 week Tai Chi exercise, the experimental group reported significantly less pain and less difficulties in activities of daily living. The Tai Chi exercise group showed significant improvement in physical functioning while the comparison group reported no change or even worse physical functioning after 12 weeks. In physical fitness test, there were significant improvements in abdominal muscle strength and balance for the Tai Chi group than the comparison group. No significant differences were found in flexibility, upper muscle or knee muscle strength.

In conclusion, Sun-style Tai Chi exercise was safely applied to the older Osteoarthritis patients for 12 weeks, and the effects on symptoms, balance, and physical functioning were supported by the results.

Disclosure:

2044

EFFECTS OF SUPERVISED AEROBIC EXERCISE IN PATIENTS WITH SYSTMIC LUPUS ERYTHEMATOSUS. Anne-Cathrine Clarke-Jenssen, Per Morten Fredriksen, Vibke Lilleby, Anne Marit Mengshoel Oslo, Norway

OBJECTIVE: To closely assess the effect of supervised aerobic exercise on disease activity, aerobic capacity, physical function, fatigue and pain in patients with systemic lupus erythematosus(SLE).

PATIENTS: Six female SLE patients fullfilling the ARA criteria, mean age(range) 47 years(39-54)and disease duration 16 years(2-34) were included.

OUTCOME MEASUREMENTS: Disease activity was assessed by SLEDAI, aerobic capacity(VO2 peak) by Naughton protocol, physical function by Modified Health Assessment Questionnaire (MHAQ with items ranged 1-4) and SF36 Health Survey(SF36 with score values ranged 1-100), and pain by SF36 bodily pain. Fatigue/vitality were assessed by Fatigue Severity Scale(FSS)(1-9) and SF36. The outcomes were assessed 3 times at baseline and at 4, 8 and 12 weeks during the intervention.

INTERVENTION: The patients performed supervised aerobic exercise by walking on treadmill at 70% of max heartbeat 40 minutes 3 times a week for 12 weeks.

ANALYSIS: Visual analysis and mean±SD for the data at baseline and during the intervention period were used.

RESULTS: Aerobic exercise was not followed by any flare of disease activity. All patients reported improved well-being after the exercise period. A variability in outcome data was observed during both the baseline and 12 weeks intervention period. Improvement in aerobic capacity, fatigue and physical function were found in 5 out of 6 patients. Three patients improved their painscores. The visual analysis may indicate that improvements in aerobic capacity and pain relief came earlier than improvements in physical function and fatigue.

CONCLUSION: Because of the low sample-size, generalized conclusion is impossible to draw. But the results are in accordance with other resent studies also showing that aerobic exercise do not worsen disease activity and can be recommended for patients with SLE.

Disclosure:

2045

MUSCLE STRENGTHENING VERSUS FLEXIBILITY TRAINING IN FIBROMYALGIA: ONE YEAR FOLLOW-UP OF A RANDOMIZED, CONTROLLED STUDY. Kim Dupree Jones, Carol S Burckhardt, Sharon R Clark, Robert M Bennett, Kathleen M Potempa Portland OR

Purpose: To evaluate the long-term efficacy of an exercise intervention for women with fibromyalgia.

Methods: The original study was a randomized, controlled, blinded intervention comparing 12 weeks of twice weekly supervised exercise consisting of either progressive muscle strengthening or flexibility training. Both groups experienced statistically significant improvements at the end of 12 weeks. However, the strength training group had a greater number and magnitude of improvements in fitness and FM symptoms. The current study was a one-year follow-up of mailed questionnaires: Fibromyalgia Impact Questionnaire, Beck Anxiety Inventory, Beck Depression Inventory, Quality of Life and the Arthritis Self-Efficacy Scale.

Results: Questionnaires were returned from 42 of 58 women (23 strength group, 19 flexibility group). Their mean age was 48.2 (SD 5.6) and mean years of FM symptoms 12 (SD 6.8). No statistically significant differences were found between groups at one year follow-up on the outcome measures. However, participants in the strength group reported exercising 4.8 times/month and those in the flexibility group reported exercising 2.1 times/month. This was an increase from the strength group's baseline in which the majority (87%) were completely sedentary ($p=0.001$). The strength training group demonstrated statistically significant within group improvements from baseline to one-year for sleep ($p=0.04$), anxiety ($p=0.05$), self-efficacy for pain ($p=0.03$) and self-efficacy for other symptoms ($p=0.001$) and number of days felt good - 2.5 days at baseline, 3.5 days one year post-intervention ($p=0.05$).

Conclusion: Women with FM who participated in 12 weeks of either muscle strengthening or flexibility training reported exercising more frequently one year post intervention than before the intervention. The strength training group maintained significant symptomatic improvements while the flexibility group did not.

Disclosure:

2046

EXCELLENT COMPLIANCE AND SATISFACTION OF RHEUMATOID ATHRITIS PATIENTS WITH A LONG-TERM HIGH INTENSITY EXERCISE PROGRAM. M Munneke, Z de Jong, AH Zwinderman, A Jansen, DCG Boonman, TPM Vliet Vlieland, JMW Hazes Leiden, Amsterdam and Rotterdam, The Netherlands

Aim: There is convincing evidence that short-lived high intensity exercise programs have positive effects in patients with rheumatoid arthritis (RA). The aim of this study was to evaluate the long term compliance and satisfaction of patients with RA with a high intensity exercise program.

Methods: 150 patients with RA who were allocated to the high intensity exercise group in a randomized trial comparing high intensity with conventional exercise, were followed over two years. All patients were encouraged to follow an intensive (75 minutes, twice a week, ± 200 exercise sessions, 2 yrs) supervised exercise program consisting of muscle strengthening and fitness exercises. Attendance over two years was recorded. After two years, satisfaction with the exercise program was examined by means of a questionnaire. Additional assessments at baseline included measures of functional ability (HAQ), quality of life (RAQOL), disease activity (DAS4) and joint damage (Larsen).

Results: Mean (SD) age and disease duration of the patients were 52.2 (11.0) and 7.4 (6.9) years, respectively. Five out of 150 patients did not start with the exercise program. After two years 118 (81 %) patients out of the 145 who initially started, still participated in an exercise class. Median attendance rate of all patients (completers and non-completers, n=145) was 74%. Worse health status as measured with the HAQ and RAQOL was weakly associated with a lower attendance rate (Spearmans rho=-0.18 and -0.25, $p<0.05$). No relation was found between the attendance rate and disease activity or joint damage at baseline. Patients were satisfied with the exercise program: 78% of the patients who started participation would (strongly) recommend participating in a high intensity exercise class to other RA-patients and 73% would like to continue participation in an exercise class after two years.

Conclusion: Compliance and satisfaction with a high intensity exercise program over a prolonged time was high. Compliance is associated with subjective measures of health status but not with objective measures.

Disclosure:

2047

FOUR YEARS OF DATA: EXERCISE EFFECTS ON MEASURES OF DISEASE ACTIVITY AND AEROBIC FITNESS AMONG RHEUMATOID ARTHRITIS CLIENTS. G Neuberger, L Aaronson, P Cagle, P Miller, S David, M Jamison, J Loudon, M Turnbull, H B Lindsley Kansas City, KS

In a study funded by the NINR (1RO1 NRO04093) and based on self-regulation, adults (n=220) ages 40-70, with RA were randomly assigned to 1 of 3 groups. Group 1 attended a low-impact aerobic exercise class 3 times weekly for 1 hour. Group 2 did the same exercises at home using a videotape, and Group 3, the control group, did not receive the exercise intervention. Measures of inflammation: erythrocyte sedimentation rate, C-reactive protein, and other measures of disease activity (walk time, left and right hand grips, morning stiffness, pain, and joint counts), aerobic fitness and psychosocial measures (fatigue, depression) were obtained. Research assistants were blinded to subjects' group assignment. Data are for baseline (T1), 6 weeks after exercise (T2), and post-intervention (T3) measures. ANOVAs for repeated measures were conducted on group means for the main effect of Time, Group, and the Interaction of Time & Group. There was a significant Time effect for aerobic fitness ($F=27.84$, $p=.000$), Right Grip strength ($F=28.42$, $p=.000$), fatigue severity ($F= 6.87$, $p= .001$), global fatigue ($F= 5.33$, $p=.006$), and tender joints ($F=7.36$, $p=.001$). There was a significant interaction effect for walk time ($F=4.52$, $p=.001$), left grip strength ($F=3.84$, $p=.004$), and pain ($F= 2.82$, $p=.025$). Groups 1 and 2 decreased the number of seconds it took them to walk 50 feet while the control increased their walk time. There were no significant interaction effects for ESR, CRP, morning stiffness, or depression. Results indicate that persons with RA can improve functional measures such as walk time, grip strength, and aerobic fitness levels by exercising without increasing measures of disease activity.

Disclosure:

2048

FMRI EVALUATION OF PAIN INTENSITY CODING IN FIBROMYALGIA PATIENTS AND CONTROLS. Masilo AB Grant, Michael J Farrell, Reshma Kumar, Daniel J Clauw, Richard H Gracely Bethesda, MD and Washington, DC

Aim: Tenderness is a defining attribute of fibromyalgia (FM). Using functional MRI (fMRI) imaging in previous studies, we found evidence that tenderness in FM results from a central augmentation of pain perception. This study used fMRI to evaluate a new cohort of FM patients and healthy controls in order to identify supraspinal structures that show graduated responses to increasing levels of painful pressure.

Methods: Pressure stimuli producing subjective levels of low, moderate and high pain were determined for 18 FM patients and 9 healthy controls. In subsequent 10min fMRI sessions, these pressures were applied in random order (25s stimulus duration, 50s interstimulus interval) to the left thumb nail bed. FMRI scans of the entire brain were obtained at 5s intervals. The data were intensity normalized, spatially smoothed and transformed into standard space.

Results: A comparison of the high pain stimulus and rest showed similar activations in both groups in contralateral primary and secondary somatosensory cortex, inferior parietal lobule, anterior insula, putamen and ipsilateral cerebellum. Correlating the three levels of painful stimuli with the fMRI signal showed similar associations in both groups in contralateral primary and secondary somatosensory cortex.

Conclusion: The results support our previous findings that similarly subjectively intense stimuli, produced by lower levels of stimulation in FM, result in comparable increases in the fMRI signal in clinical and control subjects. The correlation between stimulus intensity and cerebral response in the contralateral somatosensory regions indicates that the mechanisms responsible for pain amplification in FM preserve the pattern of stimulus intensity coding in the CNS observed in healthy controls.

Disclosure: This study was supported by Army grant 17-00-6042.

2049

SUPRASPINAL ACTIVITY ASSOCIATED WITH PAINFUL PRESSURE IN FIBROMYALGIA IS ASSOCIATED WITH BELIEFS ABOUT LOCUS OF PAIN CONTROL. Michael J Farrell, John W VanMeter, Frank Petzke, Julie M Wolfe, Masilo AB Grant, Daniel J Clauw, Richard H Gracely Bethesda, MD; Washingon, DC

Aim: Functional MRI (fMRI) can measure changes in regional cerebral blood flow associated with painful stimulation. Previous fMRI studies in fibromyalgia (FM) have indicated differences in pain processing, suggesting supraspinal pain amplification. This study examined the effect of an important clinical construct in chronic pain patients - perceived locus of control of pain - on regional blood flow patterns associated with painful pressure.

Methods: Subjects (n=21) with fibromyalgia completed the Beliefs in Pain Control Questionnaire (BPCQ). Pressure was applied to the left thumb nail bed in alternating 30 s blocks at innocuous and painful intensities for 10 min. Echo-planar images of 50 horizontal slices of 3mm thickness were obtained with a Siemens 1.5 Tesla scanner every 5 s during stimulation. The fMRI data were corrected for head motion, spatially smoothed with a Gaussian filter (FWHM= $6mm^3$) and intensity normalized. Mean differences in fMRI signal between the painful and innocuous conditions were computed and transformed into standard space for inter-subject analyses and anatomical localization. Correlations were computed at each voxel in the brain between the mean difference value and BPCQ subscale scores (Internal, Powerful Doctors, Chance). Correlations significant at $p < 0.05$ after RESEL correction for multiple comparisons are reported here.

Results: Each of the BPCQ subscale scores were related to the average increase in blood flow in discrete cerebral regions as assessed with fMRI from the innocuous to painful condition - Internal with the right SII ($r = 0.84$), Powerful Doctors with bilateral inferior parietal gyri/BA40 (Right $r = 0.82$, Left $r = 0.75$), Chance with right superior temporal gyrus (STG)/BA 22 ($r = 0.82$).

Conclusions: Endorsing a strong internal locus is associated with greater levels of pain related activity in SII possibly reflecting differential degrees of stimulus labeling and selective attention. Increased parietal activity associated with PD scores may indicate recruitment of cortico-limbic pathways involved in pain hedonics and affective responses. The implications of the relationship between C and STG activation are not readily apparent. The results of this study support the hypothesis that beliefs about pain-control influence central processing of noxious stimulation in FM.

Disclosure: This study was supported by Army grant 17-00-6042.

2050

PAIN SENSITIVITY AND BILATERAL ACTIVATION OF BRAIN STRUCTURES DURING PRESSURE STIMULATION OF PATIENTS WITH FIBROMYALGIA (FM) IS NOT MEDIATED BY MAJOR DEPRESSION (DEP). Leanne R Cianfrini, Nancy L McKendree-Smith, Laurence A Bradley, Graciela S Alarcon, Georg Deutsch, Adriana Sotolongo, Brian C Kersh, Hong-Gang Liu, James Mountz, Tykeysha Powell Birmingham, AL

AIMS: Determine whether patients with FM or chronic fatigue syndrome (CFS) differ from patients with DEP and healthy controls (HC) on measures of pain sensitivity and changes in brain regional cerebral blood flow (rCBF) during pressure stimulation.

METHOD: All subjects were right-handed women: (a) 21 with FM (ACR criteria) and no current CFS or DEP; (b) 8 with CFS (CDC criteria) and no current FM or DEP; (c) 8 with DEP (DSM-IV criteria) and no FM or CFS; and (d) 22 pain-free HC's without DEP. Subjects underwent pressure pain threshold (PT) assessment at 10 ACR tender points and HMPAO SPECT brain imaging following 5-minute (a) baseline and (b) phasic pressure stimulation of 3 right-side tender points, calibrated to individual PTs. Subjects also completed McGill Pain Questionnaire (MPQ) ratings of pressure-induced pain. Group mean PTs and MPQ ratings compared by two-tailed t-tests. Within-group changes in rCBF from baseline to stimulation analyzed by statistical parametric mapping (SPM).

RESULTS: FM patients, compared to all other groups, showed lower PTs and higher MPQ ratings following pressure stimulation (p's<.05). Both FM and CFS patients showed significant increases in rCBF during pressure stimulation in left and right somatosensory cortex (SSC) and anterior cingulate cortex (ACC) (p's<.05). HC's and DEP patients, however, showed significant rCBF increases in contralateral thalamus, SSC, and ACC (p's<.05).

	FM	CFS	DEP	HC
Pain Threshold (kg/1.54cm^2)	2.77±.13	5.98±.33	5.45±.44	5.50±.20
MPQ (Total)	40.35±3.19	25.57±3.51	28.45±4.95	23.11±2.58

CONCLUSIONS:(a) FM patients report high pain levels and show bilateral activation of brain structures that process pain when exposed to relatively low levels of pressure stimulation; (b) these responses differ from those of DEP patients and HC's, suggesting depression does not mediate pain sensitivity in FM; (c) patients with CFS and no FM do not show abnormal pain sensitivity and should be distinguished from FM patients; (d) however, it is necessary to identify factors underlying bilateral rCBF responses to pressure stimulation in CFS patients.

Disclosure:

2051

REPETITIVE MUSCLE STIMULI RESULT IN ENHANCED WIND-UP OF FIBROMYALGIA PATIENTS. Roland Staud, Kendall E Carl, Charles J Vierck, Donald D Price, Michael E Robinson, Richard L Cannon, Andre P Mauderli Gainesville, FL

Objective: Our previous wind-up experiments, using heat stimuli, have provided evidence for central sensitization in Fibromyalgia (FMS) subjects. These tests rely on nociceptive heat pulses delivered to the skin. Because most FMS subjects describe their clinical pain related to deep musculo-skeletal tissues, we tested wind-up sensations elicited from these tissues. For this purpose, we developed a computer driven, mechanical device (MD) which can deliver precise mechanical stimuli to deep tissues, using a piston with a round rubber foot plate of 0.95 cm^2. The mechanical force can be precisely set for each stimulus and registered in real time via an electronic pressure transducer.

Methods: The MD was used to measure muscle wind-up in female FMS (n=33) and normal control (NC) subjects (n=27) whose mean age was 46.5 years and 45.2 years, respectively. All subjects were trained to rate first and second pain sensations after each muscle stimulus using a well validated verbal pain (VP) scale (0 - 100). Every subject was tested with at least two series of 15 mechanical stimuli applied to the forearm using a mechanical force that elicited consistent wind-up pain. Subjects' pain ratings of every fifth stimulus were used for the calculations.

Results: FMS subjects showed temporal summation of second pain during a train of 15 stimuli (tap1: VP=14.6, tap5: VP=20.38, tap10: VP=28.46, tap15: VP=35.0) using only 2.95 lb of pessure. Testing of NC subjects with the same train of mechanical stimuli and pressure (2.95 lb) resulted in significantly lower pain ratings (tap1: VP=10.1, tap5: VP=12.7, tap10: VP=12.4, tap15: VP=15.5) (p<0.0001). In order to achieve similar wind-up ratings as FMS subjects, NC subjects required repetitive mechanical stimuli of 6.37 lb (p<0.0001)

Conclusions: We report a new method of well controlled, deep tissue stimuli for summation of second pain (wind-up). Our findings in NC and FMS subjects suggest that wind-up can originate from deep tissue structures. FMS subjects required low mechanical pressures to elicit wind-up, suggesting abnormal pain mechanisms. These same mechanisms may also play an important role in FMS pain.

Disclosure:

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GLUCOCORTICOID NEGATIVE FEEDBACK FOLLOWING INCREASED ENDOGENOUS CORTISOL AND LOW-DOSE DEXAMETHASONE IN PATIENTS WITH FIBROMYALGIA. Brent E Appleton, Christine B Brucksch, Kathryn M Dalbec, N Cary Engleberg, Leslie J Crofford

PURPOSE: Hypothalamic-Pituitary-Adrenal (HPA) axis disturbances have been demonstrated in fibromyalgia (FM). We hypothesize that differences in basal cortisol levels are due to altered glucocorticoid negative feedback. METHODS: The HPA axis response to low-dose oCRH and dexamethasone (Dex) was assessed in 11 patients with FM and in 13 age- and sex-matched controls. Subjects were excluded for active psychiatric disease and free of psychoactive medications. On day 1, oCRH (0.3 mg/kg) was administered parenterally at 8:00 pm. Blood was drawn at -15, -8, 0, +5, +15, +30, +60, +90 and +120 minutes relative to oCRH. Subjects collected saliva in the morning (AM) and evening (PM) over the next six days. Dex 0.25 mg and 0.5 mg were taken in the PM on days 3 and 5 respectively. Plasma and salivary cortisol were assayed by radioimmunoassay. Net area under the curve was determined for both ACTH and cortisol following oCRH stimulation using the trapezoidal rule. All comparisons between patients and controls were performed using t-tests.

RESULTS: There were no differences between FM patients and controls in the net integrated plasma ACTH (3811.2 ± 699.1 vs 3352.3 ± 352.0) or cortisol (2455.1 ± 255.1 vs 2493.0 ± 298.7) following low-dose oCRH. Mean baseline (days 3, 5, and 6) salivary cortisol levels were significantly lower in the AM (7.43 ± 0.56 vs 11.48 ± 0.28 mg/dL, p=0.042) and had less circadian variation (Δ4.1 ± 2.3 vs Δ9.3 ± 2 μg/dL, p=0.008) in FM patients than controls. Following oCRH stimulation testing, patients with FM but not controls had significant suppression of day 2 AM cortisol (3.34 ± 0.43 vs 7.35 ± 1.74 μg/dL, p=0.04). Following 0.25 mg of Dex, AM cortisol levels were significantly suppressed compared to baseline in controls (p<0.01) but not in FM patients (p = 0.54). Both groups suppressed following 0.5 mg of Dex. Mean PM cortisol levels were significantly higher in FM patients following oCRH (p=0.024). Mean cortisol levels rose significantly from baseline in FM patients (4.6 ± 0.9 μg/dL) while they were suppressed in controls (1.31 ± 0.18 μg/dL) on the PM following 0.5 mg of Dex (p=0.004). A loss of circadian variation occurred in patients with FM, but not controls, after either oCRH or Dex (days 2, 4, and 7).

CONCLUSION: These data demonstrate reduced circadian variation in baseline cortisol levels that is lost altogether following stimulation of endogenous cortisol or administration of Dex. These data suggest altered delayed glucocorticoid negative feedback in patients with FM.

Disclosure:

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PAIN AND DIAGNOSIS IN FIBROMYALGIA: DEVELOPMENT AND VALIDATION OF THE REGIONAL PAIN SCALE (RPS) IN 12,799 RHEUMATIC DISEASE PATIENTS. Frederick Wolfe Wichita, KS

Background: Widespread pain is a central component of fibromyalgia identification, and has been used in clinical and epidemiological studies. But there is no agreed upon method that best utilizes the information regarding pain location and intensity for the most accurate diagnosis.

Objectives: To develop and validate a measure of self-reported pain extent and location.

Methods: By a mailed questionnaire to 12,799 patients from 631 US rheumatologists, patients with RA, OA and fibromyalgia reported graded (0-3) pain in 38 articular and non-articular regions. Mokken and Rasch analysis was used to analyze the pain reports. Multiple iterative analyses identified a best 19 item 0-1 Regional Pain Scale (RPS) that was non-articular, generally monotonic, nonintersecting, had a high scalability score, and satisfied the Rasch model. Cronbach's alpha was 0.91, and the Rasch person separation statistic was 2.09 (reliability 0.81). The regions included unilateral chest, neck, upper back, lower back, and abdomen; and the bilateral regions of the upper arms, lower arms, hip regions, and upper and lower legs.

Results: The ability of the RPS to distinguish fibromyalgia from RA and OA was examined by area under the curve (AUC) measures and logistic regression. AUC values for RPS, lifetime comorbidity, and VAS pain, fatigue and sleep disturbance were 0.763, 0.744, 0.680, 0.707, and 0.691. RPS and comorbidity were not statistically different, but both differed from the other measures. In logistic regression models comparing lower and upper tertiles and quartiles, the OR for identifying fibromyalgia patients by the RPS was 10.1 (95% CI 8.1, 12.5) and 18.0 (13,5, 24.0) at tertile and quartile cut points of 8 and 10, respectively. In addition, RPS was correlated with all clinical measures of severity, with correlation coefficients between .40 and 0.58. When the RPS tertiles were studied in patients with RA, 3rd versus 1st tertiles were associated with striking increases in HAQ (0.90), pain (3.37), fatigue (3.20), sleep (3.04) (all VAS scales were 0-10), and SF-36 PCS (-9.56) scores.

Conclusions: We have developed and validated a simple regional pain score that is the best self-report measure to distinguish fibromyalgia from non-fibromyalgia patients. At high levels (8 or 10 or greater of 19), it is highly specific, and can be useful in the clinic and for screening purposes. In addition, it is a simple tool to identify RA patients with a high likelihood of also having fibromyalgia and/or very abnormal health status scores.

Disclosure: Supported by a grant from Pfizer, Inc.

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SERUM AMYLOID P COMPONENT (SAP) AND C-REACTIVE PROTEIN (CRP) OPSONIZES APOPTOTIC CELLS FOR PHAGOCYTOSIS THROUGH Fc GAMMA RECEPTORS (FcγR). Terry W Du Clos, Rebecca Baca, Carolyn Mold Albuquerque, NM

SAP and CRP are pentraxins and acute phase reactants in man and mouse, respectively. Both bind nuclear antigens that are the targets of autoantibodies in SLE and both interact with FcγR on phagocytic cells. The purpose of this study was to determine if SAP or CRP could bind to apoptotic cells prior to cell death and promote their phagocytosis through FcγR. Apoptosis was induced in human PMN and Jurkat T cells by UV-irradiation. SAP opsonization increased ingestion of apoptotic PMN by autologous macrophages. SAP and CRP increased ingestion of apoptotic, but not normal Jurkat by J-774 mouse macrophages. To determine if FcγR were involved in this ingestion, elicited peritoneal macrophages were isolated from control and γ-chain deficient mice (lacking FcγRI and FcγRIII). Both SAP and CRP increased ingestion of apoptotic Jurkat by control macrophages, but neither was opsonic when γ-chain deficient macrophages were used. Treatment of macrophages with 2.4G2 to modulate FcγRIII blocked the SAP (but not CRP) - mediated uptake of apoptotic cells. These results indicate that in the presence of pentraxins, apoptotic cells may be taken up by FcγRI or FcγRIII instead of other macrophage receptors. Ingestion through these receptors is expected to alter the pattern of cytokine production and antigen presentation. These findings may have important implications for the role of the pentraxins in host defense, inflammation and autoimmunity.

Disclosure:

2055

EOSINOPHILS CONTRIBUTE TO FIBROSIS BY INDUCING IL-6 GENE EXPRESSION IN NORMAL FIBROBLASTS. John Varga, Bruce Espenshade, Sameer Mathur, Yasuji Mori, Steve Ackerman Chicago, IL

Eosinophilic fasciitis (EF) is associated with eosinophilia and fibrosis of the subcutis, and fascia-derived fibroblasts from EF patients show evidence of activation in vitro. Eosinophils produce TGF-β, and upon degranulation release major basic protein (MBP). Although they are implicated in several fibrotic disorders, the role of eosinophils or their degranulation products in pathogenesis remains poorly defined. To characterize eosinophil-fibroblast interactions that may be involved in fibrogenesis, we examined the effects of peripheral blood-derived eosinophils, or AML14.3D10 human eosinophil-differentiated cells, on production of the potent fibrogenic cytokine IL-6 in normal lung or skin fibroblasts. Eosinophils caused marked increase in IL-6 secretion and gene transcription. IL-6 stimulation was further augmented >50% by pre-activation of eosinophils with IL-5, a potent inducer of MBP release. Increased IL-6 expression in fibroblasts was associated with enhanced NFk-B DNA-binding activity in EMSAs. Co-culture experiments using Transwell indicated that induction of IL-6 was mediated by soluble eosinophil- derived signals. TGF-β accounted for <50% of eosinophil-stimulated IL-6 secretion, as indicated by antibody neutralization. When co-cultured with eosinophils, normal fibroblasts transdifferentiated into α-smooth muscle actin-positive myofibroblastic cells. These results indicate that eosinophils induce a marked stimulation of IL-6 gene expression in co-cultured normal fibroblasts, mediated through TGF-β as well as other activated eosinophil- derived mediators. In light of the potent stimulatory effects of IL-6 on matrix synthesis, induction of IL-6 by eosinophils is likely to contribute to development of fibrosis. Furthermore, by inducing myofibroblast phenotype in fibroblasts, eosinophils further enhance the process of matrix accumulation. Eosinophils therefore may participate in fibrosis by eliciting distinct fibrogenic responses in fibroblasts through TGF-β and additional eosinophil-derived products.

Disclosure: Funded by NIAMS (AR42309) and the EMS Foundation

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EFFECT OF CPG OLIGODEOXYNUCLEOTIDES ON SYNOVIAL FIBROBLASTS. Diego Kyburz, Janine Rethage, Beat A Michel, Renate E Gay, Dennis A Carson, Steffen Gay Zurich and La Jolla, CA

CpG DNA exerts potent immunostimulatory effects on macrophages, NK cells and B lymphocytes. Recently, it has been shown that intraarticular injection of CpG oligodeoxynucleotides (ODN) can induce a transient arthritis in mice, indicating a possible role of bacterial DNA in the pathogenesis of arthritis. Whereas the activation of macrophages by CpG ODN is well established it is not known whether synovial fibroblasts are able to respond to these DNA sequences.

We studied the activation marker expression of human synovial fibroblasts in culture after incubation with CpG or control ODN in vitro. Cultured human synovial fibroblasts derived from RA patients and SV40 transformed human synovial fibroblasts from RA patients were incubated in vitro with various CpG ODN, control non-CpG ODN or without ODN. After culture periods of 24 to 48 hours the surface expression of CD106, CD40 and HLA-DR was measured by FACS using directly labeled antibodies. IL-6 levels were measured in the culture supernatants by ELISA.

Phosphorothioate as well as phosphodiester CpG ODN resulted in a significant upregulation of the surface expression of CD106 (VCAM-1) over control ODN or untreated cultures. In SV40 transformed synovial fibroblasts CpG induced up to 100% upregulation of CD106. In the tested cultured RA synovial fibroblasts the upregulation in responders ranged between 20% and 60%. CD40 was upregulated to a lesser extent in some of the tested RA synovial fibroblasts (40% upregulation in CpG treated SV40 transformed synovial fibroblasts), whereas expression of HLA-DR remained unchanged. The most potent CpG sequence was derived from Porphyromonas gingivalis DNA, which has previously been reported to be able to stimulate human gingival fibroblasts. Furthermore, IL-6 secretion was found to be increased in CpG ODN treated synovial fibroblast cultures.

These results suggest that the presence of CpG ODN derived from bacterial DNA can activate synovial fibroblasts. This activation might represent an important early step in the sequence of immune activation in inflammatory arthritis.

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2057

SOLUBLE E-SELECTIN MEDIATES ANGIOGENESIS THROUGH SRC AND PHOSPHOTIDYL INOSITOL 3 KINASE (PI3K) PATHWAYS. Pawan Kumar, M Asif Amin, Matthew A Connors, Alisa E Koch Chicago, IL

Angiogenesis, the growth and proliferation of new blood vessels, is important in a variety of pathophysiologic processes, including rheumatoid arthritis. We have previously shown that sE-selectin is an important angiogenic factor. However, the mechanism by which sE-selectin mediates angiogenesis is still poorly understood. In this study, we have analyzed the different signaling pathways by which sE-selectin mediates angiogenesis. sE-selectin induced human dermal microvascular endothelial cell (HMVEC) chemotaxis, a facet of the angiogenic response, in a concentration dependent manner in a modified Boyden chamber assay. Pretreatment of HMVECs with the Src inhibitor PP2 or the PI3K inhibitor LY294002 significantly ($p<0.05$) inhibited sE-selectin mediated HMVEC chemotaxis. However, the mitogen activated protein kinase (MAPK) inhibitor PD098059 failed to show significant inhibition. Further, we employed a Matrigel *in vitro* assay and Matrigel plug *in vivo* assay to study the role and mechanism of sE-selectin mediated angiogenesis. sE-selectin induced a 2.2 fold increase in endothelial cell tube formation in the Matrigel *in vitro* assay as compared to vehicle alone. PP2 and LY294002 significantly ($p<0.05$) inhibited sE-selectin mediated endothelial cell tube formation (47% and 49% respectively). Similarly, in the Matrigel plug *in vivo* assay, sE-selectin induced a significant increase (2.17 fold) in blood vessel formation as compared to vehicle alone. PP2 and LY294002 resulted in significant inhibition of sE-selectin mediated blood vessel growth (65% and 44% respectively). In contrast, PD098059 failed to inhibit endothelial cell tube formation in both the Matrigel *in vitro* as well as Matrigel plug *in vivo* assays, further indicating that the MAPK pathway was not involved. We next examined the signaling cascade by stimulating the HMVECs with sE-selectin for different time periods (0-30 min). sE-selectin induced a time dependent increase in tyrosine phosphorylation of proteins in the molecular weight range of Src family kinases (50-60 kDa). sE-selectin stimulated HMVECs also showed a marked increase in the phosphorylation of phosphatidyl inositol (PI) in the PI3K assay as compared to non-stimulated cells. Pretreatment of HMVECs with PP2 showed a significant decrease in PI phosphorylation, thereby indicating that sE-selectin acts through PI3K via Src kinases. Taken together, our data clearly show that sE-selectin mediates angiogenesis through the Src and PI3K pathways.

Disclosure:

2058

CARBOXYL METHYLATION OF RAS IS NECESSARY FOR PROPER RAS LOCALIZATION TO THE PLASMA MEMBRANE AND SUBSEQUENT RAS-DEPENDENT ERK ACTIVATION IN RESPONSE TO EPIDERMAL GROWTH FACTOR. Vi Chu, Kristina Loukeris, Guoming Ou, Joseph Silletti, Mark R Philips, Michael H Pillinger New York, NY

The Ras signaling pathway (Ras/Raf-1/Mek/Erk kinase cascade) is critical to cell proliferation and inflammatory responses and regulates cell types involved in rheumatoid arthritis. Ras is subject to C-terminal cysteine methylation, but the role of methylation in signaling has been unclear. To test whether Ras methylation is necessary for Erk activation we incubated Cos cells with N-Acetyl-S-Farnesyl-L-Cysteine (AFC), a methylation inhibitor. AFC inhibited epidermal growth factor (EGF)-stimulated Erk activation in a dose- and time-dependent manner (maximum efficacy at ≥12H). AFC was not toxic at doses up to 150mM. AFC did not inhibit Erk activity when added to lysates of prestimulated cells, suggesting that the target of AFC action was upstream of Erk. AFC inhibited Ras methylation, Raf-1 and Mek activation but had no effect on EGF receptor phosphorylation, confirming that the target of AFC action was Ras. Transient overexpression of the methyltransferase gene resulted in increased stimulated but not unstimulated Erk activity, suggesting that Ras methylation is necessary but not sufficient for Erk activation. Because methylation may play a role in Ras localization, we tested the hypothesis that AFC inhibition of Erk depended on the ability of AFC to cause the mislocalization of Ras. In Cos cells transiently transfected with Ras complexed to Green Fluorescent Protein (Ras-GFP) AFC blocked the movement of nascent Ras-GFP to the plasma membrane (PM) and inhibited Erk. Moreover, AFC exposure of MDCK cells stably transfected with Ras-GFP resulted in depletion of Ras-GFP already present at the PM, with kinetics consistent with those for Erk inhibition. We next engineered Ras-GFP constructs in which the Ras carboxy terminus was joined to the cytoplasmic end of the transmembrane proteins kinase-dead EGF receptor (kdEGFR) or CD8 receptor (CD8R). Transfection of Cos cells with these constructs resulted in localization of Ras-GFP to the PM in a manner unaffected by exposure to AFC. Moreover, AFC had no effect on EGF-stimulated Erk activation in kdEGFR-Ras-GFP- or CD8-Ras-GFP-transfected cells. We conclude that methylation of Ras is necessary for Erk activation, and that Ras methylation regulates Erk via its ability to effect the proper localization of Ras in the signaling pathway.

Disclosure:

2059

ATORVASTATIN PROTECTS VASCULAR ENDOTHELIUM AGAINST COMPLEMENT-MEDIATED INJURY THROUGH UPREGULATION OF MEMBRANE BOUND COMPLEMENT INHIBITORY PROTEINS. Justin C Mason, Zahra Ahmed, Anna Randi, Dorian O Haskard London, United Kingdom

Objectives: Atherosclerosis is a recognised cause of early mortality in systemic rheumatic diseases. Complement-mediated vascular injury may play an important role in the pathophysiology of this condition. As recent evidence suggests that the statins, HMGCoA reductase inhibitors, have a beneficial effect on endothelial cell (EC) function above and beyond their lipid-lowering actions, we explored the hypothesis that these drugs modulate vascular EC resistance to complement through upregulation of complement-inhibitory proteins.

Methods/Results: Human umbilical vein EC (HUVEC) were incubated with atorvastatin (1-10μM) for up to 48h and expression of decay-accelerating factor (DAF), membrane cofactor protein (MCP) and CD59 was measured by flow-cytometry. A dose-dependent increase in DAF expression of up to 5 fold was seen with maximal expression 24-48h post-stimulation. CD59 expression was also increased by upto 50% whilst MCP was unchanged. Similar results were also obtained with mevastatin (10μM). Atorvastatin-induced upregulation of DAF was dependent upon increased steady-state mRNA and de novo protein synthesis. Inclusion of L-mevalonate reversed the effect of atorvastatin on DAF expression, so confirming the role of HMGCoA reductase inhibition. Furthermore, addition of the isoprenoid intermediate geranylgeranyl pyrophosphate also reversed the effect of atorvastatin, suggesting that DAF expression is negatively regulated by geranylgeranyl synthesis. The pharmacological antagonists LY294002 and GF109203X were used to investigate the role of PI-3 kinase (PI-3K) and protein kinase C (PKC) respectively. Upregulation of DAF in response to atorvastatin was shown to be dependent on the activation of PKC but independent of PI-3K. The increase of DAF on the cell surface following atorvastatin treatment is also functionally relevant since it resulted in a marked reduction in complement-mediated EC lysis and this was inhibited by anti-DAF mAb 1H4 ($p < 0.05$).

Conclusions: These observations provide evidence for a novel cytoprotective action of the statins on vascular endothelium resulting in enhanced protection against complement-mediated injury. Modulation of complement regulatory protein expression by the statins might have direct therapeutic effects in systemic rheumatic diseases involving complement, such as SLE, and may represent a strategy by which the risk of premature atherosclerosis can be reduced.

Disclosure: Supported by the Arthritis Research Campaign (MO620).

2060

TREATMENT WITH MODIFIED SOLUBLE TACI AMELIORATES AUTOIMMUNE DISEASES BY AFFECTING HUMORAL IMMUNE RESPONSE. Kent T Miner, Susan R Eastman, Xing-Zhong Xia, Susan McCabe, Nessa Hawkins, Tom Boone, John Delaney, Francis Lee, Brad Bolon, Hailing Hsu, Sanjay D Khare Thousand Oaks, CA

Members of TNF superfamily play a vital role in various aspects related to cell proliferation and death during immune regulation. A recently discovered member of the TNF family, TALL-1 (Blys/BAFF/THANK/zTNF4) is involved in B cell proliferation and antibody production. Lupus prone NZBxNZWF1 mice at 2 months of age developed autoantibodies to ds-DNA and histone proteins when injected with recombinant soluble TALL-1 protein. TALL-1 binds to its receptors, TACI and BCMA, expressed primarily on B cells. TACI and BCMA also bind to another TNF family member, APRIL, which stimulates T and B cell responses. In animal models of lupus and arthritis, we examined a therapeutic effect of a modified soluble decoy receptor molecule. A decrease in pathogenic anti-dsDNA antibody and delayed proteinurea (>300mg/dl) was observed in lupus prone mice when treated with modified TACI. Treatment with modified TACI also prolonged the survival time. In addition to previously described lupus like disease in TALL-1 transgenic mice, approximately 40% mice developed spontaneous arthritis. Interestingly, treatment with modified TACI reduced incidence of collagen-induced arthritis in mice. Such treatment also led to a reduction in B220+ cells and related autoantibodies in animal models of arthritis and lupus, suggesting that an inhibitor blocking TALL-1 interaction with BCMA or TACI will be immunotherapeutic for antibody mediated autoimmune diseases. Our data strongly suggest that TALL-1 is an important target in B cell mediated autoimmune diseases.

Disclosure: Authors are Amgen stockholders.

2061

ADENOVIRAL DELIVERY OF TACI-FC REVERSES AUTOIMMUNITY IN MICE. Tong Zhou, Flavius Martin, Weiman Liu, Limin Zhao, Robert P Kimberly, Robert H Carter Birmingham, AL

The elevated serum levels of soluble B lymphocyte stimulator (BLyS) in murine models of autoimmune disease and in patients with systemic lupus erythematosus (SLE) and the development of serum autoantibodies and glomerulonephritis in transgenic mice over-expressing BLyS suggest an important role for BLyS in autoimmune disease. Indeed, blockade of BLyS by injection of a soluble form of TACI, a BLyS receptor, constructed as an Fc fusion protein, ameliorates kidney damage in autoimmune NZB/W F1 mice. Paradoxically, in previous studies serum levels of anti-dsDNA antibody were not reduced by administration of TACI-Fc.

To determine whether targeting BLyS could be used as an acute intervention to reverse autoimmunity, we constructed an adenoviral vector encoding a fusion protein of the extracellular domain of TACI and the Fc portion of human IgG1 (Ad/TACI). Inoculation of B6-+/+ or B6-lpr/lpr mice with Ad/TACI results in continuous, high levels of soluble TACI-Fc in the serum and inhibits antigen-stimulated antibody response. Treatment of younger B6-lpr/lpr mice with Ad/TACI, but not with a control adenovirus, prevents the development of hyperimmunoglobulinemia and blocks autoantibody production. Inoculation of older B6-lpr/lpr mice with established autoimmunity reduces serum levels of anti-dsDNA and rheumatoid factor autoantibodies. Ad/TACI treatment resulted in the loss of mature B cells, disrupted the splenic follicular mantle and reduced the number of plasma cells. A single inoculation with Ad/TACI reduced deposition of antibody in renal glomeruli. Our results suggest that blockade of BLyS is effective in the inhibition of autoantibody production and in the treatment of B cell autoimmune disease.

Disclosure:

2062

CD40-CD154 INTERACTIONS IN THE PATHOGENESIS OF MURINE LUPUS: THE BENEFICIAL EFFECTS OF EARLY AND LATE ANTI-CD154 ANTIBODY TREATMENT APPEAR TO BE MEDIATED THROUGH DIFFERENT MECHANISMS. Christopher M Burns, Sergio Quesada, Randolph J Noelle, Alan Schned Lebanon, NH

We and others have demonstrated that interruption of CD40-CD154 interactions in murine models of SLE can delay or prevent nephritis if given early in the course of the disease. Specifically, treating NZB/NZW (BW) mice with anti-CD154 antibody from age 4 to 10 months produced a 67% survival versus no survival in control groups at one year. We now report that with a minor adjustment in protocol, treatment can produce 100% survival in pre-treated BW mice at one year. More importantly, late treatment can halt or reverse disease in ~40 of mice when started after the development of significant proteinuria. Furthermore, in some mice, proteinuria could be reversed from high to negligible levels with repeated courses of anti-CD154 antibody. With this protocol, 78% of early treatment mice and 40% of late treatment mice were alive at age 18 months when the study was ended. Surprisingly, response to late therapy did not correlate with a significant reduction in anti-DNA antibodies, as had occured in pre-treatment mice. In fact, we demonstrated intense renal immune complex deposition in mice that responded to late anti-CD154 treatment and survived to 18 months. Responding mice did, however, have an altered cytokine profile compared to non-responding mice, characterized by diminished renal and splenic mRNA for IL-1 and IL-10, as well as other pro-inflammatory cytokines and chemokines, measured by Icycler (BioRad). We postulate that anti-CD154 therapy modulates disease in BW through more than one mechanism: by promoting tolerance to autoantigens when given early in the "induction" phase of the disease; and by limiting tissue damage in the kidney when given late during the "effector" phase of the disease. This suggests a role for CD40-CD154 interactions in both antibody- and cellular-mediated autoimmunity in BW. Finally, we establish that anti-CD154 treatment is also effective in NZM2.4, a substrain of BW that can be continuously bred. This last observation justifies future breeding experiments to further clarify the role of CD40-CD154 interactions in NZM2.4 and its derivative, B6.Sle.

Disclosure: Dr. Noelle is on the scientific advisory board of IDEC Pharmaceuticals, which manufactures a human version of anti-CD154 antibody.

2063

SHORT TERM COSTIMULATORY BLOCKADE COMBINED WITH CYCLOPHOSPHAMIDE DOES NOT ABROGATE GLOMERULAR DEPOSITION OF ANTIBODIES BUT ATTENUATES THE KIDNEY INFLAMMATORY RESPONSE. Jayashree Sinha, Xiaobo Wang, Weiqing Huang, Lena Schiffer, Anne Davidson Bronx, NY

It has previously been reported that a single dose of cyclophosphamide (CTX) used in combination with the costimulatory blocker CTLA4Ig can induce remission of SLE nephritis in NZB/W F1 mice. This raises the possibility that disease remission might be induced effectively and safely by short term combination therapies. In order to understand the mechanism for this effect we treated NZB/W F1 mice with established nephritis (3-4+ proteinuria) with a two week course of murine CTLA4Ig either alone or combination with anti-CD40L, with or without the addition of a single dose of CTX 750mg/m2. Remission rates as assessed by complete disappearance of proteinuria within 2-3 weeks were 0% for CTLA4Ig alone, 0% for CTX alone, 27% for CTLA4Ig/anti-CD40L, 67% for CTX/CTLA4Ig and 71% for CTX/CTLA4Ig/anti-CD40L. Mice in the CTX groups that relapsed were retreated with a second course of triple therapy and remission rates were 40%. Remissions were sustained for longer in mice treated at a younger age and in mice treated with triple therapy. Serum levels of IgG2a anti-DNA antibodies decreased by 50% only in mice treated with triple therapy but this effect lasted only 2-3 weeks while remissions were sustained for up to 20 weeks. Spleens were harvested from mice in remission at intervals after treatment. ELISpot assays revealed that the frequency of B cells producing anti-DNA antibodies decreased 3 weeks after treatment but by 5 weeks post-treatment was no different than controls. Fusions were performed and screened for anti-DNA producing hybridomas. In all cases the frequency of IgG anti-DNA antibody producing hybridomas was no different than controls. Kidneys were harvested from treated mice that were in remission (1+ proteinuria or less) 5-20 weeks after treatment. Immunofluorescence revealed intense deposits of IgG and complement in the glomeruli of treated mice but light microscopy revealed much less infiltration of inflammatory cells, absence of glomerular crescents and tubular casts and decreased mesangial proliferation. These findings suggest that costimulatory blockade together with cytoxan does not abrogate the production of antibodies that deposit in glomeruli but acts either by altering the fine specificity of glomerular binding antibodies, or, more likely, by altering the renal effector response to antibody deposition.

Disclosure: Supported by the NY Arthritis Foundation

2064

COMPLICATIONS IN THE USE OF ANGIOTENSIN RECEPTOR BLOCKERS IN THE TREATMENT OF SCLERODERMA RENAL CRISIS. Virginia D Steen, Paul J DeMarco Washington, DC and San Diego, CA

Scleroderma renal crisis (SCR) has become a treatable illness with the use of angiotensin converting enzyme (ACE)- inhibitors. Recently, newer agents, angiotensin receptor blockers (ARBs), which are very similar to ACE-inhibitors, are being used in place of ACE-inhibitors in some situations. Their effectiveness in other disease states which were responsive to ACE-inhibitors has made it reasonable to try them in SRC. Over the last year we have had 8 SRC patients who were treated with ARBs which led to uncontrolled blood pressure (BP) and/or serum creatinine (SCreat). These patients are summarized below:

Situation using ARB	BP before /after ARB	SCreat before/after ARB
ARB u2sed initially (n=3)	172/110 to 160/107	1.0 mg% to 7.0 mg%
ACE switched to ARB (n=3)	120/80 to 180/115	2.3 mg% to 3.2 mg%
ARB added to ACE (n=2)	150/85 to 150/100	3.1 mg% to 6.3 mg%

Two of the three patients who were initially treated with ARBs were switched to ACE-inhitors, but died in the next 6 months. The third patient has required dialysis in spite of going to an ACE-inhibitor. The 3 who switched from an ACE-inhibitor to an ARB did so because of side effects from the ACE-inhibitor (2 cough, 1 rash). Although BP and SCreat increased within 3 months, these were brought under control when the ACE-inhibitor was restarted. The last 2 patients, who had SRC 4 and 6 months earlier and who had a stable SCreat, had an ARB added to improve BP control. Unfortunately, both patients had increases in their SCreat and had to go onto dialysis even after stopping the ARB. We also have had 2 other patients who have been switched to ARBs because of cough but have not had any problems.

ACE-inhibitors and ARBs are very similar and for congestive heart failure and protein losing situations appear to be interchangeable. However, they may work differently in SRC which could be because the ACE-inhibitors have an effect on the bradykinin system. This may potentially play a more important role in SRC management than has been previously realized. We strongly urge patients with SRC to be treated primarily with ACE-inhibitors. Physicians should be very cautious when using ARBs in the treatment of SRC.

Disclosure:

2065

HYDROXYCHLOROQUINE IN PREGNANT PATIENTS WITH RHEUMATIC DISEASE: GESTATIONAL AND NEONATAL OUTCOME. Angela Tincani, David Faden, Andrea Lojacono, Sonia Zatti, Nicoletta Palai, Chiara Biasini Rebaioli, Micol Frassi, Marco Taglietti, Roberto Gorla, Genesio Balestrieri, Francesca Vescovi, Andrea Doria, Maria Gerosa, Laura Trespidi, Pier Luigi Meroni, Gabriella Castellino, Antonio Brucato, Gaetano Chirico, Mario Motta Brescia, Padua and Milan, Italy

Background: Hydroxychloroquine (HCQ) is widely used in pts with rheumatic diseases (RD). Because of its long half-life, discontinuing the treatment in pregnancy will not prevent fetal exposure but may precipitate flare of disease, particularly in systemic lupus (SLE). Limited data are available on the safety of the drug in pregnancy and lactation.

Objective: To evaluate gestational and neonatal outcome in pts with RD treated with HCQ during gestation and lactation.

Patients and methods: Since 1995,54 pregnancy occurred in 48 pts taking HCQ. Pts attended our Institutions because of their RD namely SLE (31), MCTD (3), primary APS (3), UCTD (6), DM (1), RA (4). All pts were taking HCQ 1 year (or more) before getting pregnant; they were kept on HCQ 200 mg/day and ASA(100 mg/day) during all the gestation and after delivery. The treatment also included corticosteroids, heparin or azathioprine when needed.

Results: 5 pregnancies (9%) aborted. In the remaining 49, the mean gestational age at delivery was 38 weeks (range 33-41). The mean birth weight of the 50 babies (one twin pregnancy) was 2951 g (range 1850-3970); 7(14%) were premature (<7 weeks) and 6(12%) were small for gestational age. No malformations were noted in the newborns; 2 urinary tract infections and 1 streptococcal pneumonia were recorded at birth. During the first month of life, 13 babies underwent ophtalmological examination: 2 presented retinal haemorrhage just after delivery, completely resolved at 1 month of age. 16 babies were discharged with breast milk feeding; lactation period ranged from 1 to 19 months. The mean follow-up time was 24 months (range 1–86); during this period all infants showed normal development and no visual or hearing abnormalities were noted. At 5 months a child suffered from Kawasaki disease and recovered in 1 week.

Comments: Our study confirms that HCQ is safe in pregnancy and provides possibly helpful data to plan the treatment of young women with RD.

Disclosure:

2066

EVIDENCE OF TRANSPLACENTAL PASSAGE OF HYDROXYCHLOROQUINE (HCQ) IN HUMANS. Nathalie Costedoat, Zahir Amoura, Guy Aymard, Du Le Thi Hong, Bertrand Wechsler, Daniele Vauthier, Marie Elisabeth Dermer, Jean-Marc Lupoglazof, Isabelle Denjoy, Yves Darbois, Jean Charles Piette Paris, France

Aim: The use of HCQ during pregnancy remains controversial (Arthritis Rheum 2000, 43 (Suppl): S272). However, there is no data about the transplacental passage of HCQ. We have addressed this issue in a prospective study in pregnant women treated by HCQ.

Methods: Eleven consecutive women treated by HCQ for SLE (n=7) or other connective tissue diseases (n=4) were included. Daily HCQ dose was either 400 mg (n=8) or 200 mg (n=3). Maternal blood collection was performed concomitantly with cord blood collection at delivery. Dosage of HCQ was assayed on whole blood. Secretion of HCQ was also measured in breast milk for 2 breast-feeding patients.

Results: HCQ was detected in all cord blood samples. Cord blood levels of HCQ ranged 303 -1580 ng/ml, with a mean +/- SD value of 894 +/- 389 ng/ml, whereas maternal HCQ concentration ranged 352-1675 ng/ml, with a mean +/- SD of 893 +/- 388 ng/ml. The mean +/- SD foeto-maternal ratio was 1.04 +/- 0.34 [range : 0.51-1.82]. Cord blood levels of HCQ were strongly correlated to maternal HCQ concentrations ($p = 0.01$ by Spearman test).

In the 2 breast-feeding mothers, HCQ concentration in breast milk was 344 ng/ml and 1424 ng/ml respectively. After correction for body weight, the daily infant intake corresponded to 0.06 and 0.2 mg/kg respectively.

Conclusion: This is the first evidence for transplacental passage of HCQ in humans. Concentrations of HCQ in cord blood samples were nearly identical to those of mothers. Though preliminary reports are reassuring, more studies are needed to ensure the absence of teratogenicity and of fetal cardiac, retinal or ototoxicity. Finally, given HCQ levels found in breast milk are very low, it does not seem logical to forbid breastfeeding when HCQ has been maintained throughout pregnancy.

Disclosure:

2067

HEPATIC SAFETY OF METHOTREXATE IN RHEUMATOID ARTHRITIS PATIENTS WITH HEPATITIS B SURFACE ANTIGEN: PROSPECTIVE ANALYSIS. Jae-Hong Park, Yong-Wook Park, Yun-Sang Bae, Tae-Hwan Kim, Jae-Bum Jun, Sungsoo Jung, Sang-Cheol Bae, Seong Yoon Kim, Dae-Hyun Yoo Seoul, Republic of Korea

Objectives: To examine the hepatic safety of the use of methotrexate (MTX), and to identify useful parameters for asessing the risk of hepatotoxicity in rheumatoid arthritis (RA) patients with hepatitis B surface antigen (HBsAg).

Patients and Methods: 19 HBsAg carriers with RA (B group) and 54 patients with RA alone (non-B group) treating with low-dose MTX, other concurrent disease modifying anti-rheumatic drug, non-steroidal anti-inflammatory drugs, steroid (prednisolone 2.5-5 mg), and folic acid, were prospectively followed up for 3 years. Liver enzymes were checked at 2-month intervals or more frequently if necessary. In B group, HBeAg, HBeAb and levels of serum hepatitis B virus DNA (HBV-DNA) were additionally checked. Changes of AST or ALT to more than 1.5 times the upper normal ranges, were considered as hepatic events. Liver biopsies was performed, and MTX was permanantly dropped out when there was a persisently abnormal ALT or AST more than 2 months after MTX withdrawal. The data were analyzed by stepwise linear and logistic regression method.

Results: We performed liver biopsy in 17 patients on B group. These cases are classed as grade I (n=8), II (n=6), and IIIa (n=3) according to the Roenigk Classification Scale. The Roenigk grade of liver histology was not correlated with hepatic event, MTX dropout, and MTX cumulative dose. Mean cumulative doses of MTX of B group with hepatic event and MTX dropout were 2,054mg and 1780mg respectively. The freqency of hepatic events (47.4% vs 7.4%, p= 0.0001) and MTX dropouts (26.3% vs 1.9%, p= 0.0008) were higher in B group compared with non-B group. HBeAg and HBV-DNA were not statistically significant. But HBsAg (+) group (n= 5) and HBV-DNA (+) group (n= 9) showed tendencies to increase hepatic events (50% vs 40%, 66.7% vs 30%) and MTX dropouts (40% vs 21.4%, 33.3% vs 20%) compared with HBeAg (-) group (n= 14) and HBV-DNA (-) group (n= 10) respectively. The serum levels of HBV-DNA showed a tendency to increase during MTX use. Ten of B group (52.6%) had significant levels of serum HBV-DNA after MTX use. Five of 7 HBeAg (-)/HBV-DNA (+) patients (71.4%) had hepatic events. 2 of these (40%) stopped taking MTX. High levels of serum HBV-DNA of MTX dropouts returned to non-detected levels after the discontinuation of MTX therapy.

Conclusion: The use of MTX in RA patients with HBsAg, is associated with a high incidence of hepatotoxicity. Only periodic measurements of ALT and AST is a valuable parameter for prediciting the risk of hepatotoxicity. HBV-DNA seems to be a useful marker to predict hepatic event and viral replication especially in HBeAg (-)/HBV-DNA (+) patients.

Disclosure:

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METHOTREXATE-MEDIATED HEPATIC FIBROSIS: ADENOSINE A_{2A} RECEPTOR ANTAGONISM DIMINISHES HEPATIC FIBROUS TISSUE FORMATION AND ENHANCES HEPATOCYTE REGENERATION. Edwin S Chan, David L Delano, Carmen Montesinos, Jhilya Mayas, Inmaculata Posadas, Samer Khaled, Scott L Friedman New York, NY

Adenosine mediates the anti-inflammatory activities of methotrexate (MTX). Various cell types, including hepatocytes, release adenosine upon exposure to MTX and/or ethanol. The observation that adenosine A_{2A} receptor agonists promote wound healing and increase dermal matrix production suggests that adenosine mediates MTX-induced hepatic fibrosis as well. We therefore determined whether adenosine might be involved in MTX-induced hepatic fibrosis in both *in vitro* and *in vivo* models.

Methods: Collagen production by rat hepatic stellate cells (rHSC) was determined as collagenase-sensitive, [C^{14}]-proline-labeled high molecular weight protein released into supernatant medium. Hepatic fibrosis was induced by injection of CCl_4 (0.05ml in oil subcutaneously 2x/wk x 4wks) in control or adenosine A_{2A} receptor-deficient mice, and mice treated with caffeine, theophylline (non-selective adenosine receptor antagonists, 25mg/kg/day in drinking water), and ZM-241385 (A_{2A} antagonist, 7.5mg/kg bid ip). Hepatic fibrosis (trichrome) was scored using Knodell grading (0-4, normal-cirrhosis), and sections were stained for proliferating cell nuclear antigen (PCNA) as a measure of hepatocyte proliferation.

Results: The selective adenosine A_{2A} receptor agonist, CGS-21680, stimulated collagen production by rHSC (19.7±6.7-fold increase, n=10, $p<0.001$). CGS-21680-stimulated collagen production was abrogated by the A_{2A} antagonist CSC (92.1±2.1% reduction, n=10, $p<0.001$), but not A_1 antagonist, DPCPX, or A_{2B} antagonist, 3-propylxanthine. Adenosine A_{2A} receptor-deficient mice were protected against development of hepatic fibrosis compared to wild-type littermate controls (Knodell grade 0 vs. 2 respectively; n=5 for each, $p<0.001$). In C57BL/6 mice, only the A_{2A} antagonist, ZM-241385, conferred any protection from hepatic fibrosis (Knodell grade 2:2:2:1; control: caffeine: theophylline: ZM-241385; n=5 for each, $p<0.001$) and significantly increased PCNA staining (1.3±0.3:1.3±0.27:0.6±0.3:4.5±0.8 PCNA+ve cells/hpf; control: caffeine: theophylline: ZM-241385; n=5 for each, $p<0.001$, ANOVA).

Conclusion: Adenosine, acting at A_{2A} receptors, may play a role in MTX- and alcohol-induced hepatic fibrosis. Moreover, blockade of A_{2A} receptors may be a novel approach to prevention and treatment of MTX-induced hepatic fibrosis.

Disclosure: Research sponsored in part by a grant from King Pharmaceutical and NIH (AR41911 and GM56268).

2069

GENOMIC SIGNATURES: GENE EXPRESSION PROFILES FOR AUTOIMMUNE DISEASES ARE DISTINCT AND DISTINGUISHABLE FROM THE NORMAL IMMUNE RESPONSE. Kevin Maas, Jason Moore, Joel Parker, Angela Slater, Sanny Chan, Nancy Olsen, Thomas Aune Nashville, TN

Autoimmune diseases are thought to arise from abnormalities of innate or adaptive immune responses. We therefore hypothesized that patients with autoimmune diseases and normal subjects responding to vaccination would show overlapping patterns of gene expression. We used cDNA microarrays to measure gene expression profiles in peripheral blood mononuclear cells from autoimmune patients with either systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) and normal individuals following routine influenza vaccination. Expression levels of over 10,000 genes and ESTs were quantitated. Three distinct clusters of genes characterized the normal immune response: an early (3 day) over-expressed cluster of 126 genes, a late (19 day) over-expressed cluster of 88 genes, and a consistently under-expressed cluster of 78 genes. The 3-day over-expressed cluster contained a number of "early response" genes. The late over-expressed cluster contained a number of genes known to play critical roles in lymphocyte function. The under-expressed cluster contained a number of genes encoding inhibitory proteins or genes which are not thought to change expression levels in response to stimulation. In the autoimmune group, we found a large number of genes (>200) that were significantly ($P<0.05$) under-expressed by at least 3-fold. There was extensive overlap between the RA and SLE patients. Fewer genes were over-expressed in the autoimmune patients, and this group exhibited greater heterogeneity than the under-expressed genes. Surprisingly, differentially expressed genes in the normal immune response group showed no overlap with those in the autoimmune disease group. Furthermore, despite the extensive overlap between RA and SLE samples, we were able to absolutely classify samples as being from pre-immune, post-immune. RA or SLE based upon results from clustering algorithms. Taken together, these findings suggest that autoimmune diseases share a common gene expression profile that is distinct from that of a normal immune response. In addition, individual autoimmune diseases have unique identifying signatures of gene expression that permit accurate classification.

Disclosure:

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DISCOVERY OF DISTINCTIVE GENE EXPRESSION PROFILES IN HUMAN ARTHRITIDES BY cDNA MICROARRAY ANALYSIS. Cornelis L Verweij, Tineke CTM van der Pouw Kraan, Floris van Galen, Ash A Alizadeh, Mike Fero, Tom WJ Huizinga, Elsbeth Pieterman, Ferry C Breedveld, Louis M Staudt, David Botstein, Patrick O Brown Amsterdam and Leiden, Netherlands; Stanford, California and Bethesda, Maryland

The clinical presentation of the chronic inflammatory joint disease rheumatoid arthritis (RA) may range from mild to severe erosive disease. Owing to the lack of knowledge of the etiology and pathogenesis of RA the current methods for classification fall short for the challenge posed by RA as a heterogeneous disease.

A powerful way to gain insight in the complex pathogenesis of RA and to classify arthritides has arisen from cDNA microarray technology, which provides the opportunity to determine differences in gene expression of a large portion of the genome in search of genes that are differently expressed between clinically diagnosed arthritides. Therefore, we studied the gene expression profile of synovial tissues from affected joints of patients with diagnosed RA (n=21) in comparison to those of patients with osteoarthritis (OA,n=9) using arrays containing 17,000 genes of importance in immunology. The results revealed 1066 genes with a two-fold difference in expression in at least 4 samples, relative to the median expression. Hierarchical cluster analysis showed a remarkable ordered variation in gene expression patterns in synovial tissues from RA and OA patients with clusters of genes having similar expression levels in clinically related synovial samples. The most prominent distinction between the two groups includes the difference in expression of genes in an adaptive inflammatory response, which are abundantly expressed in the majority of RA tissues. These clusters contain genes whose products have been implicated in RA (e.g. a T cell, B cell, MHC, activated IFNg-pathway signature) and genes not previously associated with RA (e.g Stat-1,galectins). Interestingly, OA patients and a subgroup of RA patients who lacked an inflammatory response overexpress genes involved in cell turnover, extracellular matrix remodeling and anti-inflammatory activities. This approach allowed us to i. generate a disease specific molecular signature, ii. stratify patients with RA based on molecular criteria, and iii. provide insight in the pathogenesis of arthritis.

Disclosure:

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PATTERNS OF DIFFERENTIALLY EXPRESSED GENES IN SYNOVIAL TISSUES FROM RA AND OA PATIENTS AND FROM NORMAL JOINTS. U Ungethum, D Koczan, P Ruiz, H Witt, H Huber, J Zacher, A Gursche, C Seyfert, P Reutermann, A Pruss, V Krenn, GR Burmester, St Blass, HJ Thiesen, T Haupl Berlin and Rostock, Germany

To identify patterns of genes that are specifically regulated in rheumatoid arthritis (RA) as compared to osteoarthritis (OA) and healthy (ND). Gene expression was monitored by a subtractive hybridization technique, the representational difference analysis (RDA), and by complex hybridizations on DNA chips.

Three types of subtractive hybridizations were performed: 1. OA minus RA, 2. RA minus ND, 3. ND minus OA. Difference products were cloned, sequenced and compared to published sequences (Genebank). Complex hybridizations were performed on arrayed cDNA tags (Affymetrix and RCPD) of appr. 60.000 genes. Differential expression of identified genes was validated by semiquantitative RT-PCR. Appr. 300 genes were found differentially expressed in RA synovial tissue compared to OA or ND. A selection of differentially expressed genes was identified by either method. Genes of highest regulation were associated with leucocyte, endothelial and angiogenic activation. Interestingly, a large set of genes was found downregulated in RA, such as BMP-4, IGFBP-5 and MSF. Subdividing the RA cohort by MSF expression as a measure of the protective status of the tissue, specific patterns of regulated genes could be assigned to MSF high and low expressors, respectively. Profiles of differentially expressed genes obtained by microarrays are comparable over a wide range with those obtained by subtractive hybridizations. A gene pattern is generated which is preferentially expressed in RA. Such pattern could also be of diagnostic value, especially for disease characterization.

Disclosure:

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RHEUMATOID ARTHRITIS SYNOVIUM: CLUSTER ANALYSIS AND MICROARRAY mRNA EXPRESSION PROFILES USING 23,614 GENE ELEMENTS. Ernest Brahn, Dan Fitzpatrick, Alexia Williams, Mona L Banquerigo, Kenneth Kalunian, Michael J Gresser, Carl K Edwards, III Los Angeles and Thousand Oaks, CA

Knee arthroscopy was performed on 15 patients, 8 with rheumatoid arthritis (RA) and 6 with osteoarthritis. Fresh synovia were harvested and snap frozen in liquid nitrogen. Total mRNA was isolated and amplified to define the level of gene expression using microarray analysis with three chip sets that represent a total of 23,614 elements. The osteoarthritis synovia were grouped and used as the control comparitor for each of the RA synovium. The Resolver statistical program identified which genes were significantly up- or down-regulated. Cluster analysis indicated that 2 sets of microarray profiles were present in the RA patients. The first cluster group manifested a more active and pro-inflammatory picture with up-regulated genes that included interferon gamma, IL-1 accessory protein, Il-13, stromelysin 2, cathepsin B/D, MMP 10, serine protease 11, phospholipase A2, PDGF, fibronectin, anexin A2, CD 86, HLA DR, and MHC class I. These were not statistically higher in the second cluster group although both groups shared a variety of up-regulated genes including ezrin, IL-11, and certain heat shock proteins. This data suggests that within the initial 8 patients with RA evaluated, two patterns of gene expression were identified in the target synovial tissue. These findings could be related to factors such as patient age, the duration and level of disease activity, or the use of concurrent medications. An alternative explanation is that within the clinical RA phenotype, synovitis may be driven by qualitative or quantitative differences in gene expression. With additional patients, it may be possible to identify the primary pathophysiologic processes that are critical for each patient and to specifically design the best therapeutic intervention based on that information. Subsequent samples, obtained after therapeutic initiation, might indicate whether a pattern associated with disease regression is present. Consequently, the powerful research tool of microarray expression profiling may have potentially important implications for the new field of pharmacogenomics and the future practice of clinical rheumatology.

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IMMUNOMICS IN RHEUMATOID ARTHRITIS. St Behrens, R Bergholz, F Schumann, W Schmidt, G Valet, GR Burmester, JM Engel, St Blass Berlin, Berlin-Buch, Munchen, and Bad Liebenwerda, Germany

Rheumatoid arthritis (RA) is a heterogenous disease also with respect to B- and T-cell autoreactivities: none of these is expressed in every patient and they are additionally present to a lesser extent in other autoimmune diseases. Initial data to characterize the entirety of RA-relevant autoreactivities are presented and analyzed for autoreactivites clusters that are absolutely RA-specific. Therefore, the RA-associated autoantigens citrulline, the stress protein BiP, RA33 (hnRNP A2), rheumatoid factor (RF), calpastatin (Cap) and calreticulin (Car) have either been biochemically purified, used as a kit of recombinant antigen or chemically synthesized peptides. Synovial fluids, sera and PBMCs from RA and control patients were screened for reactivity against these antigens and the data were subjected to cluster analysis. The following patterns obtained from 240 RA and 220 control patients had an RA-specificity of 100% and an RA sensitivity of 60%: RA33+RF+Cit+BiP+Cap+Car+, RA33-RF+Cit+BiP+Cap+Car+, RA33+RF+Cit+BiP-Cap-Car+, RA33-RF+Cit+BiP-Cap+Car+, RA33-RF+Cit-BiP-Cap+Car-. Classification of the entire data with CLASSIF1 revealed a positive predictive value of 94% for an unknown test set for the discrimination of RA and all control cohorts. The negative predictive value was 83%, sensitivity 91% and specificity 88%. Proteom-wide analysis of other known and unknown RA-associated autoantigens will further increase the sensitivity of the autoreactivity cluster analysis. Diagnostic confidence will markedly improve by testing autoreactivity patterns that clearly distinguish RA from other diseases. The composition of the autoreactivity patterns will also improve our understanding of RA pathogenesis.

Disclosure:

2074

APPLICATION OF DNA-CHIP TECHNOLOGY IN THE STUDY OF TRANSCRIPTOME CHANGES IN RHEUMATOID ARTHRITIS TREATED WITH A TNF-NEUTRALIZING TNF RECEPTOR:Fc FUSION PROTEIN (ETANERCEPT). Joern Kekow, Susanne Drynda, Dirk Koczan, Hans-Juergen Thiesen Gommern and Rostock, Germany

Etanercept (Enbrel) treatment induces a rapid and sustained decline in disease activity in patients with refractory rheumatoid arthritis (RA). However about 30% of patients receiving this expensive therapy are non-responders. Although the expression of single proteins, particularly cytokines and proteases, has been reported to change at the protein and mRNA levels, new methods are needed to explore the complex changes which occur after neutralisation of TNF-alpha. Microarrays are among the latest breakthroughs in experimental molecular biology. They can simultaneously monitor the expression levels of thousands of genes and produce large quantities of useful data. Here we report the application of DNA array technology (Affymetrix) to monitor changes in the expression levels of cells from peripheral blood and the synovial compartment under etanercept treatment. Mononuclear cells (PBMC) and neutrophils (PMN) from peripheral blood were prepared applying standard procedures. Synovial fluid (SF) was obtained by aspiration of knee effusions. Total RNA was isolated from PBMC, PMN and SF cells at different times during the course of treatment. Affymetrix chip technology was used to analyse the expression levels of 5600 transcripts. SF cells expressed the highest number of genes. The gene expression of cells from this compartment, however, remained nearly unchanged during the first 3 days after application of etanercept. About 3.3% and 2.4% of the assessed genes in PBMC and PMN, respectively, showed significant changes in gene expression in either direction (i.e. increase with a fold change ≥ 3, decrease with a fold change ≥ -3), whereas most changes in expression levels involved increases. Among the up- or downregulated genes were a number of genes known to be involved in pathways of transcription, apoptosis and arachidonic acid metabolism or in chemotaxis and cell adhesion as well as genes not previously examined in the context of RA. Our findings offer the first insight into the numerous secondary effects of TNF-α neutralisation. They also further elucidate the regulatory mechanisms of the immune system and the pathogenesis of RA. A chip is currently being developed which may help to monitor DMARD therapies, including biologicals.

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2075

DNA ARRAY ANALYSIS OF CYTOKINE AND CHEMOKINE-RELATED GENES IN PBMC FROM LUPUS PATIENTS IN DISEASE FLARE AND REMISSION. Violeta Rus, Sergei P Atamas, Valentina Shustova, Irina G Luzina, Barry S Handwerger, Charles S Via Baltimore, MD

Systemic lupus erythematosus (SLE) pathogenesis likely involves complex interactions between multiple cytokines, chemokines, their receptors and other immunomodulatory factors. Using DNA arrays hybridized with ^{32}PdCTP- labeled cDNA probes prepared from 2μg total RNA, we have studied the expression of 375 genes in unseparated peripheral blood mononuclear cells. In a cross-sectional study, samples from twenty-two SLE patients and eleven healthy controls were analyzed. In addition, paired samples obtained during disease flare and remission in four patients were compared. In the initial screening we selected genes that demonstrated more than 2.5 fold difference in average gene expression in patients versus controls. Only 9% of the analyzed genes met this criteria and 1.8% were significantly different by Mann-Whitney U test ($p<0.05$). Among these, IL-1R II, IL-1R AcP, IL-1β, Ciliary Neurotrophic Factor Receptor alpha (CNTF Rα), CD64, CXCR-2, TNF-related apoptosis inducing ligand (TRAIL) were higher while CCR-7 was lower in patients compared to controls. Validation studies by conventional RT-PCR were performed for four genes that have not been previously studied in SLE. Ratios between the mean gene expression in patients and controls were 1.4 ($p=0.009$) for TRAIL, 0.4 ($p=0.07$) for CCR-7, and 2.8 ($p=0.004$) for CXCR-2. Expression of CNTF Rα could not be validated by RT-PCR. When paired samples from disease flare and remission were compared, only genes expressed on both arrays were analyzed. The majority of the expressed genes did not show more than 2.5 fold difference between the paired samples. In one patient with flare consisting of joint and skin involvement no gene differed by >2.5 fold. In the other patients, different genes varied in expression in each pair of samples. CD64 and CD14 were lower during flare in a patient with arthritis, rash and leukopenia. In two patients with lupus nephritis, genes that were upregulated during flare were Nerve Growth Factor Receptor in one, and pre-B Cell Colony-Enhancing Factor, CXCR-2 and TRAIL R3 in the other. Validation studies of these differences by RT-PCR are in progress. These preliminary results suggest that DNA array technique could be used to screen for genes that have not been previously associated with SLE. If validated by RT-PCR, these genes provide excellent candidates for further studies. In addition this study underscores the significant heterogeneity among SLE patients.

Disclosure:

2076

THE IDENTIFICATION OF GENE EXPRESSION SIGNATURES IN THE PBMCs OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS. Emily C Baechler, Patrick M Gaffney, Ward A Ortmann, Franak M Batliwalla, George Karypis, Peter K Gregersen, Timothy W Behrens Minneapolis, MN and Manhasset, NY

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease with a strong genetic component. SLE is characterized by unpredictable flares of disease activity and significant patient heterogeneity in drug response, severity of disease, and clinical symptoms. Here, we report our experience using Affymetrix microarrays to identify peripheral blood mononuclear cell (PBMC) gene expression signatures specific for SLE. In a first experiment, mRNA was isolated from the PBMCs of 11 SLE patients (with low levels of disease activity) and 11 healthy, age- and sex-matched controls. RNA probes were then generated and hybridized to Affymetrix U95A GeneChips, followed by data analyses. This experiment identified 516 genes that differed significantly in their expression between SLE patients and controls ($p < 0.05$ by unpaired T test). Among these were genes involved in cytokine and immune-cell signaling, a number of interferon-inducible genes, genes involved in calcium signaling pathways, as well as genes regulating the ubiquitin pathway of protein degradation. Hierarchical clustering clearly differentiated the patient profiles from the controls, and also identified at least 2 subgroups of SLE patients. A second experiment used polyA+ mRNA isolated from purified B and T cell populations, both at rest and after in vitro activation. This experiment identified 170 genes differentially expressed in resting B cells, 421 in resting T cells, and 244 in stimulated T cells (all at $p<0.05$ level). Once again, cluster analyses completely segregated SLE patients from controls, and many of the same genes identified in the PBMC experiment were also identified here. These results demonstrate that PBMC gene expression analysis can be used to identify dysregulated biochemical pathways in SLE, and highlight the potential to identify patient sub-groups at the level of gene expression.

Disclosure:

2077

DIFFERENTIAL GENE EXPRESSION PROFILES IN DQA1*0501+ UNTREATED JDM MUSCLE:ASSOCIATION WITH INCREASED INTERFERON RESPONSE COMPARED WITH DQA 1*0501- JDM MUSCLE AND CONTROLS. Lauren M Pachman, Zivana Tezak, Marina Bakay, Jennica Lutz, Tamara O Fedczyna, Eric P Hoffman Chicago, IL and Washington, DC

Disease susceptibility for juvenile dermatomyositis (JDM), the most common of the idiopathic inflammatory myopathies, is linked to the DQA1*0501 allele. JDM symptoms often follow an upper respiratory infection.

Purpose: We performed expression profiling from muscle biopsies (MBx) of 11 untreated children with JDM (8 positive, 3 negative for DQA1*0501) to delineate the role of this DQ allele in the pathogenesis of JDM.

Methods: We compared the JDM MBx expression profiles with control MBx, using microarrays (~6000 gene Affymetrix GeneChips). We used several statistical approaches to ascertain genes specifically misregulated in each group of patients. Chip results were validated using immunostaining and real-time PCR.

Results: We found that DQA1*0501-positive JDM muscle shows high level pression of a large series of interferon response genes (MxA,6-16 gene, ISGF3,RIG-G, ISG-54), as well as high expression of cell cycle regulators p21, p27, and growth arrest and DNA-damage-inducible protein gadd45, when compared to control levels. As expected, DQ- profiles do not show such high up-regulation of interferon responsive genes. The difference in up-regulation of interferon responsive RIG-G and ISG-54 is more than 10-fold between DQ+ and DQ- JDM patients. Differential expression in DQ+ andDQ- profiles is also seen with calcitonin gene-related peptide (CGRP), which is up-regulated 10-fold in DQ+, but only 2-fold in DQ- JDM MBx and may play a crucial role in vascular component of JDM. However, the underlying relationships between the immune system, muscle fiber death, and vasculopathy in JDM are not well defined.

Summary: Based on our comparative expression profiling and confirmatory immunostaining results, we propose a pathogenic model with disease as a consequence of a persistent expression of anti-viral programs elicited by antecedent viral infection in DQ+ patients, but not in DQ- patients. Downstream events may include growth arrest in DQ+ patient smooth muscle cells, not apparent in DQ- patients. The persistent anti-viral expression profile may result in increased vascular adhesiveness, inflammation and occlusion of small vessels.

Conclusions: This model may explain the viral induction of an apparent autoimmune disease, and may have ramifications for other common inflammatory disorders of unknown etiology.

Disclosure:

2078

ANTIGEN MICROARRAY CHARACTERIZATION OF THE AUTOANTIBODY RESPONSE IN SYSTEMIC LUPUS ERYTHEMATOSUS AND RELATED DISEASES. William H Robinson, Carla DiGennaro, Wolfgang Hueber, Brian B Haab, Mark C Genovese, Sylvian Muller, Walther J van Venrooij, Josef Smolen, Patrick O Brown, Lawrence Steinman, Paul J Utz Stanford, CA; Grand Rapids, MI; Strasbourg, France; Nijmegen, Netherlands; Vienna, Austria

In systemic lupus erythematosus (SLE) and other autoimmune rheumatic diseases, our understanding of the specificity of the autoantibody response and the role of autoantibodies in pathogenesis are limited. We developed antigen microarray technology to perform multiplex characterization of autoantibody responses. Antigen microarrays are produced by applying thousands of proteins and peptides to the surface of solid supports using a robotic arrayer. We developed an array containing structurally diverse autoantigens including nucleic acids, histones, hnRNPs, snRNPs, collagens, Ro, La, SCL-70, CENP-B, Jo-1, pyruvate dehydrogenase, and post-translationally-modified antigens. We performed array analysis on serum derived from patients with SLE, rheumatoid arthritis, Sjogrens syndrome, mixed connective tissue diseases, scleroderma, myositidies, and primary biliary cirrhosis. Arrays were probed with serum from disease and control patients, followed by anti-human secondary antibodies covalently-conjugated to fluorochromes. Our array analysis identifies autoantibody response patterns characteristic of specific diseases, and we will also present array analysis of a series of SLE patients with lupus nephritis. ELISA, immunoprecipitation, and Western blot analysis validate our array results. Antigen microarrays represent a powerful tool to study the specificity and breadth of autoreactive B cell responses, and to identify candidate and define relevant autoantigens in autoimmune rheumatic diseases.

Disclosure:

2079

THE RELATIONSHIP OF SENSE OF COHERENCE AND LEARNED RESOURCEFULNESS TO FUNCTIONAL HEALTH STATUS IN WOMEN WITH SYSTEMIC LUPUS ERYTHEMATOSUS. Mary Powell Radford, VA

Guided by the Theory of Salutogenesis (Antonovsky, 1979, 1987), the purpose of this study was to analyze the relationship of sense of coherence (SOC) and learned resourcefulness (LR) to functional health status (FHS) in women with systemic lupus erythematosus (SLE). The theory frames investigations from a position of strengths and asks how persons experiencing intense stress, such as the chronicity of SLE, move toward "health-ease" rather than "dis-ease" (Antonovsky, 1993, p. 793).

The study employed a correlational design. Data were collected cross-sectionally. Instrumentation included the Orientation to Life Questionnaire, Self-Control Schedule, Arthritis Impact Measurement Scales-Revised, and Personal Profile Form. The sample consisted of 111 women (mean age = 44.9, SD = 9.6) with SLE (mean = 10.8 years, SD = 8.6).

Two major hypotheses were tested: (a) stronger SOC is related to better FHS in women with SLE and (b) higher LR is related to better FHS in women with SLE. Each major hypothesis generated eight sub-hypotheses from components of FHS (i.e., physical, social interaction, symptom, role, affect, satisfaction with current health, perception of current and future health, and overall impact of SLE).

Although the two major hypotheses were not supported, significant (p £ .003) relationship between SOC and seven FHS components were noted. Specifically, stronger SOC was noted to be related to better FHS in those seven components and higher LR was significantly (p £ .002) related to better FHS in four components.

Additional data analyses suggested SOC explained significant variability in eight FHS components (i.e., physical, social interaction, symptom, role, affect, satisfaction with current health, perception of current and future health, and overall impact of SLE). In contrast, LR was not a significant determinant of variability of FHS for women with SLE in this study.

The findings from this study suggest that the strengths SOC and LR are related to better functional health status in women with SLE. Additional study is required to determine the usefulness of those strengths in predicting better functional health status in women with SLE.

Disclosure:

2080

THE CHARACTERISTICS AND HEALTH STATUS OF PATIENTS WITH RHEUMATOID ARTHRITIS - A COMMUNITY SURVEY. Claire Bombardier, Lyn Maguire, Linda C Li, Andreas Maetzel, James Pencharz, Gwen Jansz, The Chap Team Toronto, ON, Canada

Purpose: To describe the characteristics, health status and resource utilization of patients with rheumatoid arthritis in a community-based study.

Methods: 53 rheumatologists recruited consecutive patients with rheumatoid arthritis. Patients completed a survey at baseline and 3 months asking about disease severity, health status, and health resource utilization.

Results: 253 RA patients (80% female)completed both surveys, with a mean age of 57.1 years (SD=13.3), and a mean disease duration of 11.2 years. They had on average 10.5 (SD=5.3) painful joints; 76% were moderately to severly affected by their arthritis and 54.3% reported having to cut down usual activities. Of the 42.3% who were working (part/fulltime or casual) more than one third reported difficulty at work and 20% had to take time off due to their health. Mean HAQ disability index was 1.2(SD=0.7). Mean SF36 physical component score was 32.4 (SD=10.4) and the mental component was 54.4 (SD=10.8). Patients reported an average of 4.4 (SD=2.6) comorbid conditions, mostly allergies 42.3%, headaches 32.0%, digestive problems 25.7% and hypertension 23.7%. During the six months of study, household chores were problematic, 61.5% of patients were unable to do chores, 29.8% paid others to do them and 62.3% had unpaid help; also 19.8% purchased an aid/assistive device and 10.7% used at least 1 community service. The majority of patients reported at least 1 test/investigation (95.3%), and one or more visits to a health professional other than family physician (96.8%), finally 30.4% of patients reported at least 1 hospitalization.

Conclusion: RA patients seeing rheumatologists in the community, have active disease with important limitations of function and role activities, often requiring paid and unpaid help. They also have multiple comorbid conditions and are heavy users of health care resources. These results highligh the need for a comprehensive approach to the care of RA.

Disclosure: Supported by an unrestricted grant from Merck & Co. Inc and Merck Frosst Canada

2081

SELF-CARE EXPERIENCES OF WOMEN WITH RHEUMATOID ARTHRITIS: A QUALITATIVE STUDY. Cheryl J Magnusson Vancouver, BC, Canada

Self-care has been accepted as a central mechanism by which people can enhance their capacity to cope with chronic illness and to thereby improve their quality of life and degree of independence. Understanding the individual's perspective of self-care experiences is often the missing component when health professionals plan and evaluate clinical interventions and educational activities. The individual's perspective should be an essential component of planning and evaluating activities which promote self-care behaviors and strategies for those with RA.

The purpose of this study was to gain an understanding of the perspectives of women with RA regarding their self-care experiences and to explore factors which influenced that experience.

The study explored the self-care expereicnes of women with a diagnosis of RA for at least 2 years, but no more than 5 years at the time of enrollment in the study. The participants were between the ages of 35 years and 45 years, had completed high school, and were English speaking. In-depth personal interviews were conducted with 7 women. Participants were asked to describe their health care generally and their experiences of self-care specifically. The audiotaped interviews were transcribed and analyzed using content analysis.

Analysis revealed five major themes that were incorporated into a narrative reflecting the self-care experiencs of these women: physician relationships, maintaining control, the personal need for support from others, caring for the whole self, and educating others.

The voices of these women are heard in their stories that are interwoven throughout the narrative, The findings from this study suggest ways for health professionals to assist people with RA to meet their self-care needs.

Disclosure:

2082

DEVELOPMENT OF AN INSTRUMENT TO ASSESS HOME CARE NEEDS OF PATIENTS WITH RHEUMATOID ARTHRITIS AND THEIR INFORMAL CAREGIVERS. Mirjam Galetzka, Erik Taal, Mart AFJ Van de Laar, Johannes J Rasker Enschede, The Netherlands

PURPOSE: To improve health care at home for patients with Rheumatoid Arthritis (RA), an instrument was developed to assess home care needs of patients with Rheumatoid Arthritis (RA) in relation to the help they receive from their informal caregivers and professional caregivers.

INSTRUMENT: The instrument consists of a self report questionnaire to assess health status and home care needs of patients and their informal caregivers, and a checklist that can be used by health professionals to decide about needed care facilities.

METHOD: An expert meeting (working conference) was organized to enhance the validity and practical utility of the instrument. Participants of the expert meeting were representatives of RA patients' and informal caregivers' associations and health professionals of various organizations which are involved in the care of RA patients. The participants were divided in thematic groups and asked to discuss and evaluate the content and practical utility of the instrument. Furthermore, participants received a short questionnaire to evaluate their opinions about various means of assessing home care needs of RA patients and their informal caregivers.

RESULTS: Overall, the content and practical utility of the instrument was positively evaluated. Furthermore, the expert meeting was successful in inducing manageable and clear suggestions for improvement of the quality of the instrument.

CONCLUSION: An important benefit of this approach is that during an early stage of the development process of the instrument, attention is already paid to creating favorable conditions for implementation. By means of an expert meeting, we were able to identify these conditions and to adjust the instrument to these conditions for later implementation, and thus enhance acceptance among the future users of the instrument.

Disclosure: