Objectives To confirm a hypothesis that ANKENT-triggering agents are intestinal bacteria to identify the ANKENT-triggering strains by technique of controlled association of GF mice.

Methods B10. BR males from a previously established GF colony were oligoassociated with a cocktail of anaerobic bacteria isolated from a diseased CV male. The mice were checked for ANKENT once a week. Lymphocyte phenotype in afflicted as well as healthy males was determined.

Results Oligoassociation with the selected cocktail of bacteria resulted in ANKENT in > 20% of ex-GF males, which corresponds to the situation seen under CV conditions and exceeds the ANKENT incidence in specific-pathogen-free mice.

Conclusion ANKENT-triggering agents are bacteria. The importance of viruses, protozoa and helminths in ANKENT onset can be excluded. Our findings strongly suggest that, similar to other spondylarthropathies, intestinal microflora has a inductive role in ANKENT pathogenesis.

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REFERENCE

- 1 Rehakova, et al. *Hum Immunol.* 2000;61:555

SAT0030 RELATIONSHIP BETWEEN DISEASE SEVERITY AND HLA-B27 AT TIME OF DIAGNOSIS IN ANKYLOSING SPONDYLITIS

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Background

Objectives This retrospective study was designed in order to evaluate the relationship between disease severity and HLA-B27 at time of diagnosis in ankylosing spondylitis (AS).

Methods Medical records of patients admitted to the outpatient clinic of Uludag University Medical Faculty, Department of Physical Medicine and Rehabilitation since 1988, who were diagnosed to have AS according to the 1966 New York Criteria were retrospectively reviewed. Forty-four patients were first diagnosed and had HLA-B27 evaluation. Further analysis was performed for this group which constituted the study population including sex, age at onset, disease duration until admission, HLA-B27 status, family history of AS, rheumatic symptoms, morning stiffness duration, presence of arthritis, sacroiliac tests, ESR (mm/hr), CRP (mg/dl), radiographic grade of sacroiliitis, and lumbar flexibility (assessed by Schober's test), lumbar lateral flexion limitation, finger-to-floor distance, occiput-wall distance, chin-to-manubrium distance, chest expansion.

Results Out of the study population (11 female, 33 male; n = 44) with a mean age of 34.5 ± 10.2 years, 35 patients (79.5%) were HLA-B27 (+). Mean age of disease onset was 30.0 ± 9.9 years, and mean time of disease duration until diagnosis was 55.6 ± 47.5 months. Eight patients (13.8%) had a positive family history, 9 (20.5%) had heelpain, 5 (11.4%) had chestpain, 42 (95.5%) had backpain, and 3 (6.8%) had systemic involvement at admission. Radiographic sacroiliitis was found as Grade:2 in 15 patients, Grade:3 in 16 patients, and Grade 4 in 13 patients.

Disease duration until admission was significantly less in HLA-B27 (+) patients compared to B27(-) ones ($p = 0.027$). Duration of morning stiffness was significantly higher ($p = 0.050$), and significantly more B27(+) patients had positive sacroiliac tests ($p = 0.048$). However, physical parameters such as lumbar lateral flexion and chest expansion was significantly

better ($p = 0.003$ and 0.018 , respectively) in B27 (+) patients. No significant difference was observed in other clinical and physical parameters.

Conclusion Our results suggest that B-27(+) AS patients tend to admit earlier with more severe clinical symptoms and signs before the development of physical limitations.

SAT0031 FINE EPITOPE MAPPING OF IMMUNODOMINANT PEPTIDE EPITOPES BY AFFINITY MATRIX TECHNOLOGY USING YERSINIA-HSP60-SPECIFIC SYNOVIAL CD4⁺ T-CELLS IN REACTIVE ARTHRITIS (ReA) PATIENTS

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Background A T cell response to persistent bacterial antigen is present in patients with reactive arthritis (ReA). A better characterisation of this response might lead to new treatments.

Objectives To identify immunodominant Yer-hsp60-derived CD4 T cell epitopes in Yersinia-induced reactive arthritis (ReA).

Methods In two patients with Yersinia-induced ReA synovial fluid (SF) T cell response were first investigated after *in vitro* stimulation with the Yersinia-derived protein hsp60. Fixed cells were stained with antibodies against CD4, CD8, CD69 and against the cytokine IFN γ . Patients with reacting CD4⁺ Th cells were retested and live IFN γ -secreting cells were isolated with the affinity matrix technology after short term stimulation with Yer-hsp60. Cells were expanded within 2 to 5 weeks and retested with overlapping 18 mer peptides from the Yer-hsp60 protein performing cytometric analysis of fixed cells after short term stimulation with peptided pulsed autologous PBMCs.

Results Live antigen-specific IFN γ -secreting CD4⁺ T cells were enriched to a purity of 80 to 90%. After two weeks *in vitro* expansion still 80% of the short-term cultivated T cells reacted with IFN γ -production upon Yer-hsp60 stimulation, while reaction upon stimulation with an irrelevant protein was below 1%. T cells were further expanded antigen-specifically for 3 weeks and their fine specificity was further mapped to one peptide in each patient (AA 139–156 and AA 7–30 of Yer-hsp60/GroEL). These peptides were also recognised by the patient's CD4⁺ T cells when whole blood or synovial fluid was stimulated for 6 h.

Conclusion This approach allows a sensitive and fast epitope mapping of immunodominant epitopes possibly involved in the pathogenesis of ReA.

SAT0032 PREVALENCE OF UVEITIS AND SJÖGREN'S SYNDROME (SS) IN PORTUGUESE PATIENTS WITH ANKYLOSING SPONDYLITIS

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Background

Objectives

- To evaluate the prevalence of uveitis, SS and Sicca syndrome (SicS) in patients with AS.

- To analyse the epidemiological data (Sex, age and presence of HLA B27).

Methods We included 86 patients with AS, 61 men (70.9%) and 25 women (29.1%), mean age was 48.6 ± 11.8 years (range 22–71). The disease onset had occurred at 27.4 ± 9.2 years (range 6–53). The diagnosis of SS was made according to European Criteria and all patients with SS had keratoconjunctivitis sicca.

Results Uveitis was present in 30 patients (34.9%), SS in 8 patients (9.3%) and HLA-B27 + in 63 patients (73.3%).

In the group of female patients (25) we found: 22 (88%) HLA-B27 +, 10 (40%) with uveitis, 8 (32%) with SicS and 1 (4%) with SS. In the group of male patients (61) we found: 41 (67.2%) HLA-B27 +, 20 (32.8%) with uveitis, 12 (19.7%) with SicS and 7 (11.5%) with SS.

In the group of HLA-B27 + patients (62) we found: 26 (41.9%) with uveitis and 6 (9.7%) with SS. In the group of HLA-B27 negative patients (24) we found: 4 (16.7%) with uveitis and 2 (8.3%) with SS.

Conclusion

- The prevalence of HLA-B27 was lower than in literature.
- HLA-B27 was more prevalent in female.
- The frequency of uveitis was higher in the HLA-B27 positive patients.
- We find a high prevalence of SS in male.

REFERENCES

- 1 Brito A, Figueirinhas J, Ramos M, et al. Síndrome de Sjögren. *Acta Médica Port.* 1994;7:565–76
- 2 Vaz Patto J, Figueirinhas J, Gil Forte J, et al. Sjögren's syndrome associated with Rheumatoid Arthritis. V International Symposium on Sjögren's Syndrome, Noordwijkerhout, 1995. *Clin Rheum.* 1995;14(Suppl 1):53
- 3 Figueirinhas J, Gil Forte J, Vaz Patto J, et al. Immunogenetics of Sjögren's syndrome in Portuguese patients. First International Symposium on Ocular Immunology and Inflammation, Amsterdam, 1998. *Ocul Immunol Inflamm.* 1998;6 (Suppl):68

SAT0033 ENRICHMENT OF CD8⁺ CD28- CYTOTOXIC T CELLS IN CIRCULATING LYMPHOCYTES OF PATIENTS WITH ANKYLOSING SPONDYLITIS

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Background In patients with ankylosing spondylitis (AS), HLA-B27 restricted cytotoxic T lymphocytes (CTLs) exist with specificity for arthritogenic bacteria, viral peptides or autoantigens. These MHC-class I restricted CTLs could maintain the inflammatory process even after the bacterial pathogen itself had been eradicated by antibacterial immune responses and thus be directly involved in the pathogenesis of spondylarthropathies. Phenotypically they are characterised by the distinct expression of CD28-CD57⁺CD11a^{high}.

Objectives This study was performed to directly compare the relative number of CD8⁺ CTLs from AS patients with age-matched healthy controls. Until now only few data are available for the incidence of CD8⁺CD28- T cells in autoimmune diseases.

Methods AS patients were recruited and examined at the Gasteiner Heilstollen Hospital. Controls were preselected by evaluating the proband's history and physical examination excluding an inflammatory or autoimmune diseases. Peripheral blood mononuclear cells were isolated by Ficoll density gradient

centrifugation, triple-stained for CD3, CD4, and CD28-antigens and analysed on a FACScan flow cytometer.

Results Peripheral blood was analysed from 95 AS patients and 53 age-matched healthy controls (49.1 ± 11.4 and 48.0 ± 14.0 years old, respectively). In AS patients CD8⁺CD28- T cells are expanded more than in the age-matched population ($41.2 \pm 17.7\%$ and $18.6 \pm 7.6\%$ ($p < 0.0001$), with regression lines $y = 0.23x + 29.85$ ($R^2 = 0.02$) and $y = 0.03x + 17.03$ ($R^2 = 0.004$), respectively). The percentage of CD8⁺CD28- T cells does not increase over the decades in healthy controls. Trends are described for increased percentages of CD8⁺CD28- T cells in patients with severe disease.

Conclusion These data suggest that the fraction of CD8⁺CD28- T cells is not only increased in certain infectious diseases but also in patients diagnosed with AS. Increased percentages of CD8⁺CD28- T cells during ageing might be an artificial effect of undiagnosed infectious or autoimmune diseases. This finding further supports the hypothesis that increased levels of CD8⁺CD28- T cells can be considered pathogenic, comparable to benign monoclonal gammopathy.

SAT0034 ANTINUCLEAR ANTIBODIES IN PSORIATIC ARTHRITIS

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Background It is usually believed that Antinuclear Antibodies are no more prevalent in psoriatic patients than in the general population.

Objectives To assess the prevalence of ANA positivity in a group of psoriatic arthritis patients. To search for possible differences between those who are ANA positive and those who aren't.

Methods We prospectively analysed according to a designed protocol 47 consecutive patients observed between 00/01/19 and 00/11/14. We recorded the history and physical examination with standard procedures. In all patients the presence of ANA and serum markers of viral hepatitis B and C were determined.

Results We found 38,3% of ANA positivity (Group 1 = ANA positive patients; Group 2 = ANA negative patients). The majority of group 1 patients presented a speckled pattern in title equal or superior to 1/320.

The two groups were similar in respect to gender distribution, age, duration of psoriasis, duration of arthritis, history of concomitant exacerbation of cutaneous and articular disease, history of previous or actual medication with sulphasalazine, extent of cutaneous involvement, presence of nail and scalp psoriasis and prevalence of viral hepatitis B and C.

A greater frequency of sacroiliitis (50% vs 27,6%), of family history of psoriasis (61,1% vs 48,3%) and family history of psoriatic arthritis (22,2% vs 6,9%) was seen in group 1 but didn't reach statistical significance.

Conclusion Our group of psoriatic arthritis patients shows a high prevalence of ANA positivity.

Although the frequency of patients with sacroiliitis, family history of psoriasis and psoriatic arthritis is greater in the ANA positive group we were not able to show differences with statistical significance.