Immunopathology

HARD-BOILED

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Mrs AR is a 37-year-old lady from Kenya who presented as a teenager with a history of recurrent abscesses in the axilla, breasts and groin, requiring antibiotic treatment and multiple drainages. A histological diagnosis of hidradenitis suppurativa was made, with initial good control using minocycline. Following the birth of her first child, the disease became refractory to anti-inflammatory antibiotics, anti-androgens (cyproterone acetate) and retinoic acid derivatives (roaccutane). Induction treatment with infliximab was commenced Jun–July 2014 with excellent clinical response, leading to maintenance treatment Oct 2014–Feb 2015. However, 2 months after completing the final infusion she developed new shoulder and breast lesions. Infliximab was recommenced, and she remains on 8 weekly infusions with an excellent clinical and biochemical improvement (falling CRP). Pathogenesis for this condition is unknown, but proinflammatory cytokines such as IL-1 β , IL-10 and TNF α are markedly increased in lesional and perilesional skin. Elevated serum levels of TNF α and IL-6 have also been measured in patients with stage 2 and 3 disease.

EXPLORING THE MECHANISMS OF LARGE LOCAL REACTIONS TO INSECT BITES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: There is an increased prevalence of large local reactions to mosquito bites in patients with chronic lymphocytic leukaemia (CLL), the formation of which are primarily dependent on IgE-mediated mechanisms. We hypothesise that these patients may have circulating autoantibodies that bind to the FC ϵ RI receptor of mast cells and basophils, and are capable of triggering the IgE-mediated development of large local reactions.

Methods: Six patients with CLL and large local reactions to mosquito bites were included in the study. Basophils from one atopic and one non-atopic donor were incubated with basophil stimulation buffer and serum from all six patients. Upregulation of basophil CD63 surface expression was measured.

Results: In atopic donor basophils, significant CD63 upregulation was induced by the serum of 3 of 6 patients. In contrast, no significant CD63 upregulation was induced by serum in non-atopic donor basophils.

Conclusions: Upregulation of CD63 surface expression in atopic basophil donors was detected in a subset of patients with

large local reactions to mosquito bites and CLL. This supports the hypothesis that a subset of patients with CLL may have circulating autoantibodies that bind to the FC ϵ RI receptor of mast cells and basophils, which trigger large local reactions.

WHOLE EXOME SEQUENCING IN SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS

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Systemic juvenile idiopathic arthritis (sJIA) shares clinical features with classic monogenic autoinflammatory diseases, and response to anakinra, canakinumab and tocilizumab suggests that cytokine dysregulation is a key pathophysiological mechanism. Whilst there have been HLA associations and polymorphisms noted on sJIA genome-wide association studies, in rare cases mutations have been found in genes encoding key components of the inflammatory response, which may contribute to disease pathogenesis. We aim to identify rare variants in innate immune genes that contribute to cytokine imbalance and lead to the inflammatory phenotype seen in sJIA patients by performing whole exome sequencing on probands with sJIA and their parents (trios). Recessive, compound heterozygous and *de novo* inheritance models were tested and variants evaluated for potential functional effect using a combination of SIFT, PolyPhen and MutationTaster. We confirmed candidate pathogenic variants by Sanger sequencing and cross-checked these variants with the Infevers database, known fHLH causing mutations and inflammatory pathways. We harnessed CRISPR-Cas9 gene editing technology to model mutations in appropriate cell lines, and investigate the pathogenic nature of identified novel variants. In total, there were 26 *de novo* variants (median=2, range 0–6 per trio), with 17 of these predicted to affect protein function (median=1, range 0–2 per trio). At least one *de novo* variant affects a known innate immune gene, and the pathogenic nature of this variant is being confirmed *in vitro*.

MDA-5 ASSOCIATED INTERSTITIAL LUNG DISEASE

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Autoantibodies directed against the melanoma differentiation-associated protein 5 (MDA-5) have been recently described in association with clinically amyopathic dermatomyositis (CADM) and rapidly progressive interstitial lung disease.¹ Here we present the clinical features, diagnostic approach and the management of a child with an MDA-5 autoantibody detected by line immunoassay.

The patient is a 2-year-old boy born to non-consanguineous Chinese parents. He presented with a one week history of fever and cough with lobar consolidation demonstrated on chest X-ray. This presentation was on the background of a six month history of low-grade fevers, refusal to weight-bear and small self-healing ulcers of the hands, ears and axilla. Skin biopsy demonstrated non-specific fat necrosis. He rapidly deteriorated during the first week of his admission with increasing oxygen requirements necessitating intubation and ventilation, and bilateral interstitial lung changes. Lung biopsy demonstrated acute diffuse alveolar damage. Electron microscopy showed prominent tubuloreticular structures in the endothelial cells. He was extensively investigated, with the pertinent findings including a 1:2560 nucleolar pattern ANA and a positive MDA-5 antibody on line blot. He was treated with high-dose methylprednisolone, plasmapheresis, rituximab and cyclophosphamide.

Several discussion points arise from this case presentation, including the presentation of dermatomyositis in the paediatric population, investigation of children with autoimmune disease, and the management of severe interstitial lung disease in this context.

Reference

1. Muro Y, Sugiura K, Hoshino K, *et al.* Epidemiologic study of clinically amyopathic dermatomyositis and anti-melanoma differentiation-associated gene 5 antibodies in central Japan. *Arthritis Res Ther* 2011; 13: R214.

TRIPLE THREATS: TRACKING ANTIGEN-SPECIFIC T CELLS IN A CASE OF CONCURRENT AUTOIMMUNITY, INFECTIOUS DISEASE AND POSSIBLE MALIGNANCY

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We present a case of a 50-year-old man who presented with newly diagnosed HIV infection and a progressive jejunal/omental infiltration causing subacute ileus. He had been diagnosed with celiac disease one year previously. He had a prominent CD8 T cell lymphocytosis demonstrated in peripheral blood, omentum, bone marrow and lymph node biopsies demonstrated two clonal T cell receptor (TCR) gene rearrangements on capillary electrophoresis. Extensive investigations for HIV associated infections were negative. We undertook TCR sequencing using deep parallel sequencing and found oligoclonal TCRs, including one which corresponded to a recently described CD8 $\alpha\beta$ TCR stimulated by *in vivo* gluten challenge in patients with celiac disease. This case provides novel insights into celiac disease pathogenesis and demonstrates the potential of high dimensional methods in characterising the nature of T cell-mediated pathologies at the single cell level.

CEREBROSPINAL FLUID FREE LIGHT CHAIN QUANTITATION IS A SPECIFIC MARKER FOR INFLAMMATORY CENTRAL NERVOUS SYSTEM DISORDERS IN A PAEDIATRIC PATIENT COHORT

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Cerebrospinal fluid (CSF) analysis is routinely used in the diagnostic work up of a range of inflammatory, infective and congenital neurological conditions. This study aims to investigate the utility of CSF free light chain (FLC) analysis in a paediatric population with central nervous system (CNS) disorders. Paediatric CSF samples were analysed for the presence of kappa and lambda FLC as assessed by two nephelometric methods, with results correlated to clinical diagnoses. FLC were also compared to currently used CSF diagnostic tests including oligoclonal bands (OCB), CSF IgG and albumin levels, and differential cell count. Of 70 samples analysed, 29 (41%) had an inflammatory or infective diagnosis and 41 (59%) presented with a range of non-inflammatory aetiologies. OCB was detected in 4 (6%) subjects (sensitivity 9.4%, specificity 97.6%). Thirteen (19%) patients had elevated kappa or lambda FLC on the IMMAGE 800, of these 12 (92%) had an inflammatory disease (sensitivity 37.9%, specificity 97.6%). On the BN II 24 (34%) patients had elevated results, of which 21 (88%) had an inflammatory disease (sensitivity 72.4%, specificity 92.7%).

The results of this study support the use of CSF FLC analysis for the detection of intrathecal immunoglobulin synthesis in the diagnosis of paediatric CNS inflammatory conditions.

BROAD SPECTRUM OF IMMUNE AND NON-IMMUNE PATHOLOGY IN INHERITED DEFECTS OF STAT SIGNALLING

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Numerous monogenic diseases associated with mutations leading to either enhanced or impaired STAT activity have been identified. Each disease leads to a variety of phenotypes which shed light on the functions of these proteins, their roles in regulating each other, and, perhaps most importantly, lend to potential treatment modalities. While the list is growing, loss-of-function (LOF) mutations have been identified in STAT1, STAT2, STAT3 and STAT5b, while gain of function (GOF) mutations have been identified in STAT1 and STAT3. STAT1-LOF can lead to mycobacterial infection and, along with STAT2LOF, viral infection as well. STAT3LOF leads to severe staphylococcal infection of the skin and lungs, marked IgE elevation, and, along with STAT1GOF, mucocutaneous candidiasis and a variety of vascular, bone and other connective tissue abnormalities. STAT3GOF, like STAT5b loss of function patients, have short stature, lymphoproliferative disease, autoimmunity and infection. Study of these clinical phenotypes have yielded interesting insights into fundamental pathways, such as