

The Histiocyte Society 23rd Annual Meeting

Cambridge 23-25 september 2007



Inledning

Detta är en sammanfattning av det tjugotredje årliga mötet i den internationella Histiocytosföreningen på University Arms Hotel i Cambridge den 23-25 september 2007. Närvarande var sammanlagt över 100 läkare, forskare och föräldra-representanter.

Konferensen innehöll studiepresentationer inom sjukdomsgrupperna LCH, FHL och HLH, samt grundforskning inom relevanta områden för dessa sjukdomsgrupper. Många betydelsefulla resultat visades.

Det är mycket information man får under dessa tre dagar och skulle jag framhålla något som är mer intressant för oss i Sverige, så är det naturligtvis resultat där Jan-Inge Henter och andra svenska läkare och forskare varit med i bilden. Som vanligt tillhör de studierna frontlinjen i forskningen och utvecklingen av nya metoder. Dr Servet-Delprats studie om LCHs biologi är också något man inte bör hoppa över. Hennes forskning, tillsammans med bl a Jan-Inge Henter och Selma Olsson, kan få en stor betydelse för den framtida utvecklingen.

Dr. Vasanta Nanduris studie av livskvaliteten på lång sikt hos patienter som har eller haft LCH är också intressant och inte heller så svår att förstå.

Men vad man är mest intresserad av avgörs naturligtvis av vilken sjukdom och vilka problem ens eget barn eller anhörig lider av. I år har vi fått tillgång till alla studiernas engelska utdrag och dessa finns bifogade sist i den här rapporten. Jag tror att ni som är intresserade av speciella ämnen kan hitta dessa utifrån rubrikerna i de engelska utdragen.

Om ni har några frågor är ni välkomna att höra av er till föreningen så ska vi försöka svara så gott vi kan.

Urban Beinö

Representant för den svenska Föräldraföreningen för Histiocytos i Cambridge 2007

MÖTET ÖPPNAS

Jan-Inge Henter hälsar alla välkomna till årets konferens. Han tycker att Cambridge är en mycket bra plats att hålla konferensen. Det kommer bli 83 Nobelprisvinnare från Cambridge berömda universitet, vilket är flest i hela världen. Det är första gången som mötet hålls i Storbritannien. Däremot hölls ett av de första planeringsmötena för att bilda the Histiocyte Society i Cambridge. Så nu är cirkeln, på sätt och vis, sluten.

Under åren har stora framsteg gjorts när det gäller förståelse och behandling av HLH. Nu menar han är det dags för LCH. Vägen till LCHs biologi öppnar också för många andra forskningsfält. Han ser optimistiskt på framtiden och tror att det kommer att bli en spännade konferens.

University Arms Hotel





Innergården vid King's College

GÄSTTALARE

Det var fem gästtalare i år. (De finns utförligt presenterade längre fram i samband med de engelska utdragen.) Två berörde LCH, två HLH och en talade om EUs regelverk för att driva kliniska försök.

Om LCH

Christine Servet-Delprat från Frankrike presenterade en mycket intressant studie som kan ha en avgörande betydelse för förståelsen av LCHs biologi.

Vasanta Nanduri från Storbritannien talade om permanenta skador av LCH och hur det påverkar livskvaliteten hos patienter på lång sikt.

Om HLH

Michael Jordan från USA. Immunregulerande rubbning vid HLH.

Gillian Griffiths, Storbritannien. Ökad förståelse för vad som biologiskt händer vid olika HLH-varianter.

EUs regelverk för kliniska försök

Linda Porter, Storbritannien.

INTERNATIONELLT FÖRÄLDRAMÖTE

På kvällen, den första konferensdagen, hölls ett föräldramöte där styrelsen för the Histiocyte Society, förutom Lisa Filipovich, berättade och svarade på frågor.

De flesta föräldrarna kom från Storbritannien – England, Skottland, Irland – men där fanns också föräldrar från andra länder som Tyskland, Kanada, USA och Spanien.

Vi delades in i två grupper; de som var intresserade av HLH följde med Jan-Inge Henter och Jim Whitlock och de som var intresserade av LCH satt tillsammans med Nicole Grois, Vasanta Nanduri och Carlos Rodrigues-Galindo, vilken var den grupp jag följde med.

Först hade Carlos Rodrigues-Galindo en genomgång om sjukdomen, hur den kan se ut, diagnos, symptom och behandling. Sedan blev det tid för en lång frågestund och diskussion. Nicole Grois, som är ansvarig för LCH - CNS studiegruppen i Wien svarade på många frågor som rörde CNS engagemang och problem kring detta. Det var många som hade barn med CNS-relaterade problem. Vasanta Nanduri hade många svar som rörde olika komplikationer och sena effekter av sjukdomen. Alla tre läkarna tog sig verkligen tid att besvara våra frågor och gjorde det med stort intresse och engagemang.

Mötet den här kvällen var väldigt givande och en fanatstisk möjlighet för oss föräldrar att ställa frågor direkt till experterna. Stämningen var avslappnad och gemytlig men

samtidigt mycket koncentrerad. Många var oroade över sjukdomens effekter och förlopp och hade angelägna frågor kring sina barns situation. Det var slående hur olika patienter och familjer drabbas av LCH. Naturligtvis finns det många likheter, men av oss som deltog här var det ingen som hade en likadan sjukdomshistoria.

Alla som var med hade stort utbyte av kvällen. Även läkarna som på det här sättet också får mer information och kunskap om hur det kan vara för olika patienter världen över.



Del av stads kärnan i Cambridge

FÖRÄLDRAMÖTE I THE HR TRUST'S REGI

The HR Trust hade ordnat ett särskilt föräldramöte den andra dagen, som tyvärr krockade med flera intressanta studiepresentationer på konferensen som jag valde att lyssna till istället. Men jag deltog senare på eftermiddagen. Tre brittiska läkare och forskare var med; Kevin Windebank, Peter Beverly och Jane Salotti samt Richard Price från HR Trust som är förälder till ett barn med LCH. Mötet var väldigt informellt och det var

även en hel del barn med, vilket var roligt att se. Det bildades spontant grupper av föräldrar och anhöriga som diskuterade sina erfarenheter. Det var intressant att höra vad andra föräldrar hade för frågor och erfarenheter. En slutsats man kan dra av allt som kom fram under eftermiddagen är att vi i Sverige är mycket lyckligt lottade, inte bara när det gäller sjukvård, utan även när det gäller saker som resurser för barn med speciella behov, skola, uppföljning, tillgång till experter och läkemedel mm.

MÖTET AVSLUTAS

Jan-Inge Henter avslutar mötet med att betona två saker:

1. Att förstå LCH kan öppna dörrar till andra forskningsområden, och man är genom gemensamma ansträngningar på god väg.
2. Utan föräldragrupperna skulle dessa möten inte kunna hållas. Det gäller både det praktiska arbetet kring själva arrangemangen men också det samarbete som finns mellan patienter, föräldrar och läkare.

Han tackade för det fina arrangemanget och för banketten i anrika King's College där bl a några ur King's College berömda kör sjöng före och under middagen. Detta var en stor upplevelse och en bra avslutning på hans sista år som president för the Histiocyte Society. Nu blir det Lisa Filipovich från USA som tar över i de kommande tre åren.

Nästa konferens kommer att hållas i Berlin den 1-3 oktober 2008.

Vi hoppas att nya intressanta forskningsresultat kommer att presenteras då!

King's College och River Cam



KEYNOTE GUEST SPEAKERS



Christine Delprat was born in Toulouse (F) in 1967. Selected by French national championship (1988) to be trained in Ecole Normale Supérieure, she completed graduate programs in Molecular Biology, Biochemistry, Microbiology and Human Physiology (ENS Cachan-Paris and University of Paris 7), including success at the French national championship of Aggregation of Biochemistry (1991) and a Ph.D. in Immunology (1995) under the supervision of Dr Jacques Banchereau (Schering-Plough Dardilly and University of Lyon) on the mechanism of IgG subclasses isotype switching in human B cells. She carried out postdoctoral work at Ecole Normale Supérieure de Lyon with Pr Chantal Rabourdin-Combe (1996-98) on the study of measles virus-infected human dendritic cells (DC) and obtained a permanent position of Associate Professor of Immunology at the University of Lyon in 1998. Dr. Delprat has been involved in Immunology education for over one decade. Her research focus is the DC plasticity in acute (viral infections) or chronic (Mycobacteria infections, Rheumatoid arthritis) inflammatory microenvironment and specifically, these last years, the ability of DC to undergo cell fusion and develop novel effector functions such as bone resorption. Following the 15th Nikolas Symposium (2005), she devoted part of her research time to explore the plasticity of human DC in LCH lesion through collaborative efforts involving Prs Maurizio Arico, Jan-Inge Henter and Maarten Egeler. Find her 21 publications in major international journals through Pubmed with "Servet*C"[Author] or "Delprat*C"[Author] or "Servet*Delprat*C"[Author].



Linda Porter, PhD, Senior Trials Manager, Children's Cancer and Leukaemia Group; Senior Lecturer, Department of Cancer Studies and Molecular Medicine, University of Leicester. Linda has been managing clinical trials for 18 years. She started as a post-doc at Nottingham University looking at the effect of disease on targeted-release radiolabelled GI dosage forms. She spent 8 years working for PPD Development, a Contract Research Organisation running healthy volunteer clinical trials for the pharmaceutical industry. She was based at the 50-bed Phase I Unit in Leicester where she project managed studies ranging from the simple large cohort crossover food effect or drug interaction pharmacokinetic studies, to those of a first-in-man, multiple rising-dose, safety and tolerability design. Linda has also worked as a clinical projects manager for Toyama Europe, managing their out-sourced early phase trials running internationally outside of Japan. She was responsible for overseeing the CROs running the trials that were designed to establish the safety and efficacy of NCEs being developed for neurology and rheumatology indications. Linda now works as senior trials manager for the Children's Cancer and Leukaemia Group (CCLG), a non-commercial organisation running paediatric oncology clinical trials. She is based at the CCLG Data Centre which is part of the Department of Cancer Studies and Molecular Medicine at the University of Leicester where she is employed as a senior lecturer. CCLG currently has a portfolio of 60+ trials. These are designed to improve survival where current prognosis is poor, or to maintain high cure rates where current treatment regimens are good whilst reducing adverse or late effects of treatment, or to determine the biological effects of diagnostic or prognostic factors, as well as the testing of new agents in children.



Dr Michael Jordan graduated from the University of Texas Southwestern Medical School in 1993. After completing a pediatrics residency at the Children's Hospital of Dallas in 1996, he moved to Denver, Colorado for a fellowship in Pediatric Hematology/Oncology at The Children's Hospital. After completing clinical training, Dr Jordan joined the laboratory of Drs Philippa Marrack and John Kappler in 1997, where he studied basic T cell biology. In 2002, Dr Jordan became an Instructor of Pediatrics and in 2003 was named Assistant Professor at the University of Colorado Medical School. In 2004 he moved to Cincinnati Children's Hospital/University of Cincinnati, where he joined the divisions of Immunobiology and Hematology/Oncology. During the last 3 years he has established an independent scientific laboratory and has continued to care for children with histiocytic disorders. Dr Jordan's laboratory is currently focused on understanding the biology of HLH and LCH, with the goal of developing novel therapies for these disorders. His laboratory also maintains basic scientific interests in immune regulation, tumor immunology, and vaccine therapeutics.



Dr Vasanta Nanduri is a Physician in the Department of Pediatrics at Watford Hospital and Great Ormond Street Hospital for Children in London, UK. She entered medical school at the age of 16 and completed her medical education, post graduate degree in Pediatrics, and residency in India. Dr Nanduri relocated to England and trained in pediatrics and specialized in Pediatric Oncology and Endocrinology at the Great Ormond Street Hospital and University College Hospital in London. She soon became intrigued by histiocytic disorders after meeting Dr Jon Pritchard. Dr Nanduri is especially interested in the late effects of Langerhans cell histiocytosis and how it relates to a patient's quality of life and published her thesis on this subject. She has attended the Histiocyte Society Annual Meetings since 1995 and currently serves on the Histiocyte Society Executive Board. She is a member of the Late effects, CNS and Epidemiology groups of the Histiocyte Society. She has had over ten peer reviewed articles and over twenty abstracts published in the field of histiocytosis. Dr Nanduri has also been honored by both presenting and being an invited guest speaker at several national and international meetings.

Gillian Griffiths began her scientific career as a PhD student with Cesar Milstein, showing that somatic mutation lead to affinity maturation of the immune response. As a post-doctoral fellow at Stanford, she became interested in the cell biology for cytotoxic T lymphocytes and pursued this research on becoming a member of the Basel Institute for Immunology. Her lab focused on the use of genetic diseases to identify proteins required for secretion funded by the Wellcome Trust in Oxford. She has recently moved to Cambridge as a Wellcome Trust Principal Research Fellow. Gillian Griffiths has been elected as a Fellow of the Academy of Medical Sciences and EMBO.

LONG-TERM COURSE OF HYPOTHALAMIC PITUITARY TUMORS (HPT) AND NEURODEGENERATION IN LANGERHANS CELL HISTIOCYTOSIS (LCH).

Bernhard Fahrner, Daniela Prayer, Martha Wnorowski, Helmut Prosch, Milen Minkov, Helmut Gadner, Nicole Grois
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Introduction: Involvement of the hypothalamic-pituitary (HP) axis is the most frequent CNS manifestation in LCH and often leads to diabetes insipidus (DI) and anterior pituitary hormone deficiencies (APD). The associated MRI findings range from a loss of the normal signal in the posterior pituitary, a thickening of the infundibulum, to profound tumors (HPT). The long-term course and the influence of therapy have not been studied in detail yet.

Material and Methods: We investigated 22 patients, registered at the LCH study center, with HPTs defined as mass lesions >6.5mm, who were investigated with median 6 (2-17) MRI studies over an observation period of median 6y (8m-14y). They were 14 males and 8 females. Their age at the diagnosis (Dx) of LCH was median 5y (1m-28y). In 2 patients the HPT was detected before LCH Dx, in 20 patients median 2y 7m (2w-11y) after LCH Dx. LCH directed therapy was given to 19 patients prior to HPT Dx and included LCH standard therapy (n=18), 2CDA (n=1), Indomethacin (n=2), 6MP/MTX (n=8). At the HPT Dx the size of the HPT ranged from 6.6mm to 25mm (median 11.5mm), 18 patients had already DI and 14 APD. In 8 patients neurodegenerative (ND) lesions in the cerebellum or basal ganglia were seen (=radiological ND), 3 of these had neurological symptoms. Treatment for HPT included LCH standard therapy (n=9), 2CDA (n=7), Indomethacin (n=5), 6MP/MTX (n=8) and irradiation (n=5) given for variable periods and with more than one regimen applied in most patients. The response of the tumor size was variable. After HPT Dx 1 patient developed DI after 3m, and 4 patients APD after median 1y (10m-2y5m). Radiological ND became evident in further 14 patients after median 3y 6m (5m-11y6m), 2 of them had progressive neurological symptoms.

Summary: All 22 patients with HPT > 6.5mm had endocrine deficiencies, and all had radiological ND after HPT Dx. In none of the patients hormone deficiencies and ND regressed or resolved under therapy. 5 ND patients were reported with clinical ND with ataxia, dysarthria, behavioral disturbances and intellectual impairment, 2 of them had symptoms before the LCH Dx, in 3 patients symptoms developed after LCH-standard like treatments.

Conclusion: From these data it appears that therapies applied for the treatment of LCH and HPT did neither prevent hormone deficiencies nor the development of radiological ND. To adequately judge the influence of therapy on the course of ND careful long-term monitoring of HPT patients with serial MRI accompanied by neuropsychological tests is necessary.

COMPARISON OF FDG-PET SCANS TO CONVENTIONAL RADIOGRAPHY AND BONE SCANS IN MANAGEMENT OF LANGERHANS CELL HISTIOCYTOSIS.

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PURPOSE: To evaluate effectiveness of FDG-PET scans in identifying sites of active disease and assessing response to therapy in patients with Langerhans cell histiocytosis (LCH). LCH is characterized by lymphocyte proliferation with infiltration of bone, skin, thymus, lymph nodes, spleen, bone marrow, lung, pituitary, or brain. We hypothesize that FDG-PET imaging, which identifies metabolically active cells, is superior to conventional radiography (plain film, CT, MRI) and bone scans in identifying LCH lesions. Additionally, changes in standardized uptake value (SUV) in serial studies of a lesion can indicate increased or decreased activity of the cells within a lesion before changes are evident by plain films or bone scans, giving early indication of response to therapy or recurrence of disease.

METHODS: One hundred and two FDG-PET scans for 44 patients (41 children, 3 adults) with biopsy-proven LCH were reviewed and 83 were compared with corollary imaging modalities. These 83 scans were rated for overall clinical utility: false positive or negative ("false"), confirming lesions identified by another imaging modality ("confirmatory"), or showing new lesions, response to therapy or recurrence of disease activity ("superior"), in comparison to plain film, CT, MRI or bone scans.

RESULTS: FDG-PET was rated confirmatory or superior in 79/83 (95%) of reviewed scans. FDG-PET scans proved superior in 32/83 (39%) cases by detecting new lesions, recurrence, or response to therapy via change in SUV before other radiographic changes, and confirmed reported disease locations in 47/83 (57%) of comparisons. FDG-PET was rated superior to 4/13 (31%) bone scans, 9/19 (47%) MRI, 5/28 (18%) CT, and 14/23 (61%) plain films, and confirmatory to 9/13 (69%) bone scans, 7/19 (37%) MRI, 23/28 (82%) CT, and 8/23 (35%) plain films. Regarding individual lesion evaluation, FDG-PET was rated confirmatory or superior in 39/42 (93%) pelvic lesions, 7/7 (100%) rib lesions, 60/62 (97%) other bone lesions, 67/70 (96%) skull lesions, 36/51 (71%) vertebral lesions, and 18/21 (86%) other body sites (brain, liver, lungs, lymph nodes, and soft tissue).

CONCLUSIONS: Whole body FDG-PET scans can often detect LCH activity and early response to therapy with more accuracy than other imaging modalities in patients with LCH lesions in the bones and soft tissues. Whole-body FDG-PET scanning is an important and informative study at diagnosis and for following disease course in patients with LCH.

HEMATOPOIETIC CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING (RIC HCT) FOR HEMOPHAGOCYTTIC LYMPHOHISTIOCYTOSIS (HLH) AND X-LINKED LYMPHOPROLIFERATIVE SYNDROME (XLP).

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Introduction: The largest published studies of HCT for HLH have reported early treatment related mortality (TRM) of approximately 30%. In an attempt to reduce early TRM and ultimately improve long term survival, we initiated a pilot study using a RIC HCT protocol in 2006.

Materials and Methods: Ten patients with HLH (4 mos. to 12 years) or XLP (1.5-16 years) were prepared with Campath, Fludarabine and Melphalan prior to infusion of either matched sibling donor (2) or closely matched unrelated donor (8) marrow. Two patients were at very high risk due to short gut syndrome or prior liver transplant. Three patients had been treated for one or more lymphoid malignancies.

Summary: There were no complications with the conditioning protocol and all patients initially showed 98-100% donor chimerism in the peripheral blood. Over the first few months chimerism dropped in 6/7 patients with HLH. Despite discontinuation of immunosuppressive therapy in the 6 patients, chimerism continued to decline to or below 60% in 4 patients (60%, 30%, 18%, 0.2%), who then received therapeutic T cell infusions (stored at time of HCT). Three of these four patients developed grade 2-3 acute GvHD which required rehospitalization. However, all recovered and, currently, donor chimerism for the entire patient group ranges from 69-100% (median 100%). Six of the ten patients are off all immune suppression and all are free of their underlying disease. Median survival post HCT is currently 10 months and all patients are alive.

Conclusion: This pilot study illustrates the potential for reducing early TRM of HCT for hemophagocytic disorders, while emphasizing the importance of preparing for management of unstable chimerism in the early posttransplant months. Longer follow up is needed to evaluate impact on disease free survival.

MICROARRAY ANALYSIS OF GENE EXPRESSION IN CD207+ AND CD3+ CELLS FROM PARIETAL LANGERHANS CELL HISTIOCYTOSIS LESIONS.

Carl E. Allen, Liunan Li, Eastwood Leung, M. John Hicks, Sivashankarappa Gurusiddappa, Sergery Torsky, and Kenneth L. McClain

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Introduction: Langerhans Cell Histiocytosis (LCH) is caused by pathological proliferation of the Langerhans cell, a dendritic cell normally restricted to the skin. LCH can arise in virtually any organ system, and uncontrolled multi-system LCH is often fatal. LCH lesions are heterogeneous, including infiltrating T cells. The microenvironment of the Langerhans lesion has been characterized as a "cytokine storm" with high levels of proinflammatory cytokines. In this study, we isolated CD207+ LCs and CD3+ T cells from LCH lesions in order to determine cell-specific gene expression profiles which may elucidate the etiology of the pathologic pro-inflammatory environment in LCH lesions.

Methods: Biopsy samples from freshly-excised parietal LCH lesions from three patients were processed, and CD207+ and CD3+ cells were isolated by FACS. RNA was isolated from the cells, quality was verified by the Agilent 6000 Pico LabChip, then cDNA was generated, amplified, fragmented and biotinylated. cDNA probe was also generated in parallel from CD207+ cells isolated from 30 human foreskin samples and from CD3+ cells isolated from 20 human tonsil samples. The experimental and control cDNA probes were then hybridized to Affymetrix U113 Plus 2.0 Array chips which contain targets for over 47,000 transcripts. Chips were scanned with the GeneChip Scanner 3000. The tonsil pool CD3+ chip was used as the reference for the LCH CD3+ samples. The skin pool CD207+ chip was used as reference for the LCH CD207+ samples. Spot intensity was normalized to housekeeping genes, and data were analyzed with BRB Array Tools 3.5. All genes over-represented or under-represented greater than 4-fold in all three CD3+ and CD207+ samples were identified.

Summary: Compared to the LC isolated from skin, in the LCH CD207+ cells 67 genes had decreased expression (>4-fold in all three samples) and 76 had increased expression. Down-regulated genes in the LCH CD207+ cells included regulators of cell adhesion and apoptosis. Up-regulated genes in the LCH CD207+ cells included TNF family members and genes involved in Notch and Ras signaling pathways. Compared to the CD3+ cells isolated from tonsils, in the LCH CD3+ cells 17 genes had decreased expression and 46 had increased expression. Up-regulated genes included TNF family members, genes involved in NF- κ B signaling, and genes involved in cell-cycle regulation. Interestingly, many pro-inflammatory genes previously associated with LCH lesions were not identified in these experiments.

Conclusion: Gene expression analysis of CD207+ LC and CD3+ cells from LCH lesions reveals distinct profiles. Gene expression and signal transduction pathway activation specific to cells in LCH lesions will provide better understanding of the etiology of this disease as well as potential targets for therapy.

**MECHANISMS OF DENDRITIC CELL-DERIVED
GIANT CELL FORMATION AND LCH.**

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A striking feature of Langerhans cell histiocytosis (LCH) lesional tissue is the presence of multinucleated giant cells (MGC) whose origin is unknown. These lesions are enriched with dendritic cells (DC), resembling Langerhans cells (skin DC), but exhibiting an aberrant monocyte-macrophage-DC (CD14⁺ CD68⁺ CD1a⁺) semi-mature (CCR6⁺ MHC-II^{low} CD40^{high}) phenotype. In 2004, we published that immature DC transdifferentiate into functional osteoclasts (OC) in the presence of M-CSF and RANKL (Blood. 2004;104:4029-4037). Transdifferentiation operates through fusion of intermediate adherent bipolar fusiform mononuclear cells expressing CD14, CD1a, and RANKL and able to induce T cell proliferation. In 2007, as previously described for human DC, we demonstrate that murine DC, either in vitro generated from Flt3-positive bone marrow progenitors or ex vivo purified from spleen, are able to develop into OC in response to M-CSF and RANKL, in vitro (Eur JI. 2007;37:747-757). This transdifferentiation is driven by the immune environment that controls DC maturation, cell fusion, tartrate-resistant acid phosphatase (TRAP) and bone resorption activities. Only immature myeloid DC have the capacity to become OC since mature myeloid DC or plasmacytoid DC do not. As in human, additions of the pro-inflammatory cytokines, such as IL-1beta and TNF-alpha, or human rheumatoid synovial fluid, increase murine DC transdifferentiation into OC, whereas IFN-alpha inhibits it. The adaptive cytokine IFN-gamma inhibits DC fusion while IL-4 increases it. IL-2, IFN-gamma and IL-4 inhibit TRAP and bone resorption activities contrary to IL-10 which enhances both activities. Our data therefore suggest that DC-derived OC may be directly involved in the osteolytic lesions observed in human inflammatory bone diseases such as rheumatoid arthritis or LCH. We then studied the ability of monocyte-derived DC from LCH patients to transdifferentiate into OC. We noticed an overexpression of TRAP activity in DC and DC-derived OC from LCH patients. In LCH, as in other granuloma diseases including MGC such as Mycobacterium infection or Crohn's disease, the lesional microenvironment is enriched in IFN-gamma, a powerful inhibitor of DC transdifferentiation into OC. Trying to understand the molecular basis of these last findings, we discovered a novel pathway of DC fusion, highly potentiated by IFN-gamma. Importantly, this novel fusion activity, quantified in LCH serum, correlates with disease activity. Thus, this novel DC fusion pathway represents a major pathogenic mechanism whose targeting may have clinical value in the treatment of LCH and other granulomatous inflammatory disorders.

HLH: A DISORDER OF IMMUNE REGULATION.

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Precise control of the immune response is critical because effective responses to pathogens may be both life-saving and quite toxic to the host. The importance of this precision is illustrated by the human disorder hemophagocytic lymphohistiocytosis (HLH). Individuals born with a variety of genetic mutations affecting perforin-dependant cytotoxic function may experience dramatic and overwhelming episodes of immune activation, which define HLH. Similarly, perforin-deficient mice have been recognized to develop an HLH-like disease process following infection with lymphocytic choriomeningitis virus (LCMV). The basis for this abnormal immune activation is not defined, though deficient cytotoxic elimination of infected cells has been presumed to underlie this disease process.

We have sought to define the pathophysiology of HLH and understand how cytotoxic function exerts an immunoregulatory effect, by studying LCMV-infected cytotoxic-deficient mice. Experimental evidence will be presented which defines a feedback loop involving dendritic cells, T cells, and cytotoxic function. Our data suggest that T cells 'prune' dendritic cell populations via perforin-dependant cytotoxic killing, and thereby modulate the amplitude of immune responses. Our data also indicate that ongoing T cell/dendritic cell interactions during the peak of the effector T cell response have a previously unappreciated importance.

Though immune hyperactivation appears to underlie HLH, it is still not clear how this abnormal response leads to the clinical features of HLH. Furthermore, while it has long been assumed that macrophages play an important role in the development of HLH, no experimental evidence has ever proven this point. As a first step towards understanding this process, we previously determined that interferon gamma (IFN-g) is a critical mediator of HLH-like pathology in LCMV-infected mice. More recently, we have found that administration of IFN-g is sufficient to mimic many of the clinical features of HLH in normal mice. Experimental evidence will also be presented which establishes a clear causal role for macrophages and hemophagocytosis in the development of HLH disease features. Finally, studies exploring the biology and mechanism(s) of hemophagocytosis will be presented.

ALTERATION OF THE IMMUNOLOGICAL SYNOPSIS IN HLH.

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Cytotoxic T lymphocytes and Natural Killer cells use specialised secretory granules, termed lytic granules, to destroy virally infected and tumorigenic targets. The secretion of these granules is focused at a precise point of contact within the immunological synapse formed between killer and target. In order to determine the proteins required for secretion from CTL and NK cells my laboratory has studied cells derived from patients in which CTL and NK secretion is disrupted, including many patients with Lymphohistiocytosis. Using this approach we, and others, have shown that the lytic granules move along the microtubule cytoskeleton towards the immunological synapse and are then captured and 'docked' at the membrane at a specialised secretory domain using AP-3, RILP, Rab27a, Munc 13-4 and Lyst. We have determined the sequence of events and used this information to identify new proteins in the sequence of events which lead to secretion and function of the CTL.

EARLY ONSET OF FHL3 IS SIGNIFICANTLY ASSOCIATED WITH DISRUPTIVE *MUNC13-4* MUTATIONS.

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Introduction. Mutations in the *Munc13-4* gene have recently been described in patients affected by familial hemophagocytic lymphohistiocytosis defined as FHL3. Due to limited number of patients reported so far, only little information is available on the genotype-phenotype correlations in this subset of patients.

In this study we have analyzed our cohort of patients with FHL3 to establish the correlation between the functional impact of the mutation with the age at the onset of the disease.

Methods. We sequenced from genomic DNA the 32 exons and the adjacent intronic regions of the *Munc13-4* gene in all patients with HLH, diagnosed according to current criteria, in whom *PRF-1* mutations were not detected. All patients identified with documented biallelic mutations, confirmed also on the parents, were diagnosed as FHL3. Mutations leading to premature stop or to sequence frameshift were defined as disruptive, while those associated with single nucleotide change were defined as missense. This information was related to the age at the diagnosis of FHL. Statistical significance was tested by Student's T test.

Results. Of the 29 patients with FHL3, 16 (55%) had two disruptive mutations, 6 (21%) had two missense mutations, and the remaining 7 had one disruptive and one missense mutations. The age at the diagnosis ranged between 1 month and 17.8 years, with a median of 6.4 months. The 16 patients with two disruptive mutations had a median age at diagnosis of 3 months (range, 0.9-11.7), which was significantly younger than that of the 13 patients with at least one missense mutation (median age 94.7 months; range, 1 m to 17.8 years) ($p < 0.001$). When compared with the age at diagnosis of 16 patients with FHL2 and two disruptive mutations, we found that FHL2 had a significantly earlier onset ($p < 0.001$).

Conclusion. The age at the onset of FHL3 is significantly younger in patients with two disruptive *Munc13-4* mutations not allowing residual protein activity. Otherwise, in patients with two missense mutations allowing residual protein function, the disease onset may be delayed, up to adolescent or even young adult age.

MUTATIONS AFFECTING MRNA SPLICING ARE THE MOST COMMON GENETIC DEFECTS IN PATIENTS WITH FHL3.

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Introduction. Mutations in the *Munc13-4* gene have recently been described in patients affected by familial hemophagocytic lymphohistiocytosis defined as FHL3. Among the reported mutations, a few are responsible for impaired splicing of the *Munc13-4* gene. They are located in the exon/intron boundaries or in the U1 recognition exon site.

The role of mutation(s) causing alternative splicing sites is currently a focus of investigations in different human disease, with a growing number of disease-related mutations directly linked to aberrant splice process. Notably, for some genes such as *NF1* and *ATM*, nucleotide substitutions affecting the splicing process may account for up to 50% of all pathogenic mutations. In this study we document that *Munc13-4* mutations leading to splicing errors represent a large proportion of mutations observed in FHL3.

Methods. We sequenced from genomic DNA the 32 exons and the adjacent intronic regions of the *Munc13-4* gene in all patients with HLH, diagnosed according to current criteria, in whom *PRF-1* mutations were not detected. To better understand the role of nucleotide substitutions predicted to affect splicing, we cloned and sequenced cDNA and RNA from some selected cases.

Results. We identified 33 families with FHL3. Of them, 21 (64%) had at least one mutation responsible for a splicing error. We observed a total of nine such different mutations: of them only two had been previously reported by other groups, two were included in our recent report (Santoro et al., *JMG* 2006), while five had never been described before.

Conclusion. Splicing errors are frequently observed in patients with FHL3. Since mutation analysis restricted to the coding exons may overlook such pathogenic mutations, this finding has implications in designing the strategy of analysis of the families with suspected FHL.

DEFECTIVE CYTOTOXIC LYMPHOCYTE DEGRANULATION IN SYNTAXIN-11-DEFICIENT FAMILIAL HEMOPHAGOCYTTIC LYMPHOHISTIOCYTOSIS 4 (FHL4) PATIENTS.

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Introduction: Familial hemophagocytic lymphohistiocytosis (FHL) is typically an early onset, fatal disease characterized by a sepsis-like illness with cytopenia, hepatosplenomegaly and deficient lymphocyte cytotoxicity. Disease-causing mutations have been identified in genes encoding perforin (*PRF1*/FHL2), Munc13-4 (*UNC13D*/FHL3), and syntaxin-11 (*STX11*/FHL4). In contrast to mutations leading to loss of perforin and Munc13-4 function, it is unclear how syntaxin-11 loss-of-function mutations contribute to disease.

Material and methods: Cytotoxic lymphocyte function was assessed in FHL patients (n = 5) with mutations in *PRF1*, *UNC13D*, and *STX11*, respectively. Polarization of perforin in NK cells, degranulation in NK cells and T cells triggered by different activating stimuli, and peripheral blood lymphocyte lysis of K562 target cells was analyzed and compared to healthy, age-matched controls. Lymphocyte function was also assessed after activation for 72 hours with IL-2

Summary: Freshly isolated, resting natural killer (NK) cells and CD8⁺ T cells express syntaxin-11. In infants, NK cells are the predominant perforin-containing cell type. NK cells from FHL4 patients fail to degranulate when encountering susceptible target cells. Unexpectedly, IL-2-stimulation partially restores degranulation and cytotoxicity by NK cells, which could explain the less severe disease progression observed in FHL4 patients, compared to FHL2 and FHL3 patients.

Conclusions: Since the effector T cell compartment is still immature in infants, our data suggest that the observed defect in NK cell degranulation may contribute to the pathophysiology of FHL, that evaluation of NK cell degranulation in suspected FHL patients may facilitate diagnosis, and that these new insights may offer novel therapeutic possibilities.

SYNTAXIN-11 EXPRESSION AND ITS ROLE IN PRIMARY MONOCYTES: IMPLICATIONS FOR FAMILIAL HEMOPHAGOCYTTIC LYMPHOHISTIOCYTOSIS.

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening inflammatory disorder classified into two forms, familial HLH (FHL) and secondary HLH. FHL is an autosomal recessive disorder and disease causing mutations in the *STX11* gene, encoding syntaxin-11, have recently been identified in a subset of FHL patients. However, the biological function of syntaxin-11 in the immune system and its potential role in FHL development is still largely unknown. Pathologically, an accumulation of activated macrophages and CD8⁺ T cells is one hallmark of FHL, suggesting a role of monocytes/macrophages in FHL development. FHL is also characterized by hypercytokinemia with elevated levels of TNF-alpha, interferon-gamma (IFN-γ), and other cytokines. In the current study, we investigated the expression of syntaxin-11 in monocytes as well as the Fc-receptor (FcR)-mediated cytotoxicity and phagocytic activity of syntaxin-11 deficient monocytes from FHL patients with *STX11* gene mutations (FHL4) and from healthy controls after knocking down syntaxin-11 expression by the specific short interfering (si)RNA method.

Materials and Methods: Three FHL4 patients were studied. Mutations in the *STX11* gene were identified by direct DNA sequencing. Syntaxin-11 expression was evaluated by Western blot. FcR-mediated cytotoxicity of syntaxin-11 deficient monocytes was determined by an ⁵¹Cr-release assay using adherent cells or purified monocytes selected from three FHL4 patients and healthy controls after knocking-down syntaxin-11 expression as effectors (E) and sheep red blood cells (SRBC) labelled by ⁵¹Cr as target cells (T) in the presence of an anti-SRBC IgG antibody. The siRNA gene silencing technique was employed to generate syntaxin-11 deficient monocytes purified from healthy blood donors by a monocyte negative selection kit.

Results: Our results demonstrate that primary monocytes from healthy controls abundantly express syntaxin-11, whereas the monocyte cell lines THP-1 and U937 express much lower levels. Moreover, monocytes (adherent cells) from peripheral blood of two FHL4 patients, carrying *Del110C* and *Q268X* mutations respectively, yielded truncated syntaxin-11 products as visualized by Western blot. Interestingly, the expression of syntaxin-11 in monocytes from healthy controls was up-regulated by IFN-γ stimulation in a dose- and time-dependent manner. Furthermore, the FcR-mediated cytotoxicity against SRBC by the syntaxin 11 deficient monocytes from three FHL4 patients and three healthy controls after knocking down syntaxin-11 expression were comparable to those of relevant controls (40% vs. 35% and 35% vs. 42%, at E/T ratios of 0.5, respectively). Functional studies on the phagocytic activity of syntaxin-11 deficient monocytes generated by the syntaxin-11 siRNA silencing approach are ongoing.

Conclusions: Syntaxin-11 is abundantly expressed in primary monocytes. *STX11* mutations result in truncated syntaxin-11 protein expression in monocytes. Studies on the role of syntaxin-11 in monocytes/macrophages are ongoing.

HEALTHY CARRIERS OF PERFORIN H222Q HAVE REDUCED NK FUNCTION AND DECREASED PERFORIN CONTENT BUT THE PROTEIN DETECTED IS THE MATURE PERFORIN ISOFORM.

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Introduction: Prior studies of human perforin in primary and transformed NK cell lines have demonstrated that native human perforin is synthesized as a precursor protein, with subsequent maturation to a functional isoform. Our lab has recently demonstrated that recombinant human perforin introduced into rat basophilic leukemia (RBL) cells also undergoes maturation, and that both precursor and mature bands are detectable by Western blot analysis of whole cell lysates. The perforin missense mutation-H222Q has been identified in several patients with HLH, and our previous studies in RBL cell lines, as well as new studies in CD8 T cells from perforin knockout mice (unpublished results), demonstrate that this mutant perforin fails to show the characteristic banding pattern on Western blot analysis. Instead, high molecular weight protein aggregates are detected. We hypothesized that these protein aggregates result from protein misfolding and that misfolded protein may also be present in human lymphocytes from heterozygous individuals carrying the mutated allele, leading to reduced perforin protein and function. The purpose of this study was to characterize the endogenously expressed perforin of 3 family members heterozygous for the H222Q mutation by flow cytometry, NK function, and Western blot analysis.

Materials and Methods: DNA sequencing of the perforin gene in an infant presenting with HLH revealed her to be homozygous for the H222Q substitution, while the healthy mother, father and sibling were noted to be carriers. Perforin expression was analyzed by flow cytometry and NK function assayed by chromium release from K562 target cells (Cincinnati Children's Hospital Diagnostic Immunology Laboratory). Cellular lysates were derived from freshly isolated peripheral blood mononuclear cells (PBMC) from the patient's sibling, mother, and father, and adult controls. Western blots were performed on non-reduced protein lysates following SDS-PAGE, and probed with P1-8 mAb. Due to severe lymphopenia, the patient's cells were not analyzed.

Summary of Results: Family members heterozygous for H222Q perforin exhibited decreased expression in NK cells by flow cytometry and low NK function. However, mature perforin was readily detectable by Western blot of the PBMC cell lysates of the human controls and H222Q carriers. Also noted were additional bands consistent with degraded protein. Lysates from RBL cells and murine "knockout" CD8 cells expressing perforin-H222Q displayed protein aggregates, but there were no aggregates detected in the PBMC lysates from the 3 carriers of the H222Q mutation.

Conclusion: Native human perforin, though decreased in cellular amount, is fully processed to the mature form in lymphocytes in the peripheral blood of individuals heterozygous for the H222Q mutation. Notably, misfolded protein aggregates are not seen. This suggests that the mutated H222Q protein may undergo rapid elimination in the native cytotoxic lymphocyte and likely has no impact on the proper folding and maturation of the wild type protein.

GRANZYME B EXPRESSION IS A BIOMARKER OF CYTOTOXIC LYMPHOCYTE ACTIVATION IN HLH AND FACILITATES THE DIAGNOSIS OF PERFORIN DEFICIENCY WHEN MISSENSE MUTATIONS ARE PRESENT.

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Introduction: Perforin and granzyme B are constitutively expressed in human natural killer (NK) cells, allowing fully functional killer cells without activation. Cytotoxic T cells (CTL) acquire expression of perforin and granzyme B during activation and differentiation from naïve CD8 cells. Studies in humans have shown that levels of perforin and granzyme B in cytotoxic lymphocytes increase in various clinical scenarios such as septic shock and active systemic lupus erythematosus. We hypothesized that levels of granzyme B may increase in NK and CD8 cells as a result of immune activation in all children with hemophagocytic lymphohistiocytosis (HLH), while elevated perforin levels would only be seen in children without perforin mutations.

Material and methods: As an international reference lab for the diagnosis of HLH, we have analyzed NK function, perforin and granzyme B expression in cytotoxic lymphocytes by flow cytometry, and genetic defects in perforin and Munc in hundreds of patients by previously reported methods.

Summary of Results: Granzyme B expression is increased in NK cells in children presenting with HLH due to perforin defects, Munc defects, and HLH with no known defects in either gene. In patients with HLH without evidence of perforin deficiency, perforin and granzyme B expression are correlated in both NK ($R^2=0.34$, $p<.003$) and CD8 cells ($R^2=0.51$, $p=.0001$). In patients with known perforin mutations, perforin expression is typically dramatically reduced or absent, while granzyme levels are increased. However, several patients have been identified with missense mutations in perforin who by flow cytometry appeared to have only slightly low or even normal perforin expression in NK cells with dramatic elevations of granzyme B. Knowledge of the correlation between perforin and granzyme levels alerted us that the perforin levels in these patients were "falsely normal," and sequencing identified these individuals as heterozygous carriers of A91V and R33H; and individuals homozygous for R356W, T435M/V453M (present on the same allele), and R225W.

Conclusions: DNA sequencing remains the gold standard for diagnosis of genetic defects in PRF1, but detection of perforin by flow cytometry is often the first test ordered when evaluating patients suspected to have primary HLH. Assessment of granzyme B levels in NK and CD8 cells identifies immune activation in HLH and facilitates the diagnosis of perforin deficiency when perforin levels are "falsely normal".

INVESTIGATOR RESPONSIBILITIES AND THE PRACTICAL REQUIREMENTS FOR RUNNING CLINICAL TRIALS IN CHILDREN.

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Clinical trials performed in children should be carried out under conditions providing the best possible protection for this vulnerable population, whilst recognising that children have the right to benefit from research. Investigators involved in interventional clinical trials carried out under the provisions of Directive 2001/20/EC need to be aware of the additional ethical and practical implications of involving children in research.

The EU 'paediatric regulation' 1901/2006 came into force in the European Union on 26 January 2007 (<http://www.emea.europa.eu/htms/human/paediatrics/regulation.htm>). The aim of this legislation is to improve health among children in Europe through measures designed to stimulate the development of new medicines for use in the paediatric population, to ensure that they are appropriately tested and authorised, and to improve the availability of paediatric-relevant information about the use of the medicines in the published Summary of Product Characteristics (SmPCs).

This regulation was drawn up in response to the fact that ca50% of medications given to children in hospital are prescribed 'off label' i.e. outside the SmPC, and therefore without supporting evidence of the safety and efficacy in children. During childhood the development of organ systems such as liver, kidneys and lungs, means that children of different age groups handle drugs differently and their clinical response may differ from that of adults. Children may be dosed ineffectively or may be overdosed. Off-label use can also result from the fact that many marketed drugs are not available in an appropriate formulation.

The investigator must ensure that the trial is appropriately designed taking into consideration ethical issues, assessment of risks and benefits, and research practicalities that are particularly important for a paediatric study: The number of children used should be the minimum within statistical constraints of achieving a valid result; There should be direct benefit to the child, unless there is likely to be significant benefit to other children with the condition and a minimal risk and burden on the participants; There is an obligation to minimise pain, distress, fear and practical impact of the study on the family; It should be conducted in specialist centres with paediatric experience; The formulation and route of administration must be acceptable to children – is the taste bitter, or the tablet hard to swallow, or the drug given by needle? The therapeutic window of the drug, and mechanism for metabolism and excretion, must be considered; Recognition and assessment of SAEs in children is complex; Validated biomarkers as surrogate endpoints may be needed.

Informed consent is a particular challenge for paediatric trials. Information must be given at a level appropriate for the child's age and development. Consent is actually obtained with each visit for children, because if assent is withdrawn the child must be allowed to drop out from the study regardless of parental consent unless the condition is life-threatening.

These investigator responsibilities will be discussed in terms of a practical GCP approach to ensure the successful running of clinical trials in children.

LONG TERM SEQUELAE AND QUALITY OF LIFE AFTER LANGERHANS' CELL HISTIOCYTOSIS.

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Langerhans' cell histiocytosis can involve several organs/ tissues and may result in permanent sequelae in 20 to 50% of patients, especially those with multisystem disease.

Orthopaedic disabilities: The bony skeleton is the most common site of involvement of LCH. Reconstitution and modelling of bones in children often improves outcome. However bony damage may result in pathological fractures, limb deformity, scoliosis and vertebra plana. Facial and skull abnormalities may require reconstructive surgery. Mastoid lesions can result in deafness and tooth loss may require orthodontic surgery.

Skin: Scarring can be seen at sites of LCH skin involvement, and from surgical procedures.

Endocrine sequelae

Diabetes insipidus: The posterior pituitary is particularly susceptible and diabetes insipidus (DI) can occur in up to 50% of patients. It can precede the diagnosis of LCH or appear years after LCH is diagnosed. **Anterior Pituitary dysfunction:** Growth hormone deficiency is the next most common endocrine abnormality, occurring in up to 20% of patients. Other hormone deficiencies are less common. Pituitary radiation does not cure DI, and may result in anterior pituitary damage and should be avoided. Growth may also be affected by other factors such as bony deformity, steroid therapy and the effects of the disease itself. Children with hypothalamic damage have multiple hormone deficiencies, and may also develop the "hypothalamic syndrome", which includes aggressive behaviour, eating disorders, obesity and temperature instability. Rarely, the thyroid gland may be directly affected by LCH.

Lungs: Although acute lung involvement may occur in up to 50% of children with multisystem LCH, permanent lung damage is less common, possibly due to repair of alveoli in the young child. As smoking has been shown to be the most important risk factor for worsening lung disease, patients with LCH should be strongly recommended to refrain from smoking.

Liver: Progressive liver damage may result in sclerosing cholangitis and cirrhosis quite early in the disease course. Sclerosing cholangitis is often fatal and liver transplantation might be the only cure.

Brain: Neurological problems such as cerebellar ataxia, psychological problems and learning difficulties can develop several years after LCH. The natural history of CNS disease is variable and may remain stable or progress resulting in severe disability. MRI scan of the brain may show bilateral cerebellar signal change, but may not correlate with clinical features. Neuropsychological sequelae include learning deficit, loss of memory, poor school performance, and emotional disturbance and may result in handicap.

LCH and Malignancies: LCH seems to be associated with malignancy more often than would be expected by chance. In general, there appear to be two patterns of association between LCH and malignancy: ALL usually precedes LCH, whilst AnLL and solid tumours develop after LCH.

Summary: LCH can cause significant long term morbidity resulting in disability in survivors affecting the ability to lead a normal life. Carefully planned, regular long-term follow up is essential for all patients 'cured' of LCH to ensure that sequelae are recognised early and appropriate interventions made to improve the patients' "Quality of Life".

GENE EXPRESSION PROFILING IN FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS REVEALS DIFFERENCES AMONG THE VARIOUS GENETIC FORMS OF THE DISEASE.

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Introduction: Familial hemophagocytic lymphohistiocytosis (FHLH, MIM 267700) is a rare, life-threatening, autosomal recessive, immune disorder, which results from genetic defects in the regulatory pathways that normally maintain immune homeostasis and execute the natural termination of effector immune responses. The common pathway of FHLH pathogenesis is the expansion and persistence of cytokine-secreting immune cells, which would normally be removed by NK cells. FHLH is genetically and clinically heterogeneous. Three autosomal recessive gene defects underlie 40-50% of primary (familial) cases worldwide: perforin (20-30%, FHLH2), the major immune cytotoxic protein, MUNC 13-4 (20%, FHLH3), a protein involved in exocytosis of perforin-bearing cytotoxic granules during apoptosis and STX11 (FHLH4), member of soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNARE). Thus in more than half of the FHLH cases, the affected individuals have other as yet unknown genetic alterations. Considering the genetic heterogeneity of FHLH we hypothesize that gene expression profiles will reveal significant differences between the genetic subgroups of FHLH, will identify useful diagnostic, prognostic and pathogenesis-related biological markers of the disease and provide new insights into the genes and pathways critical for NK and CTL cell function in vivo.

Material and methods: Twenty-two patients with various genetic forms of FHLH were included in the study. Nine of the twenty-two patients (FHLH2) carried homozygous or heterozygous inactivating mutations in their *PRF1* gene. The remaining patients had unknown cause of the disease. Peripheral blood mononuclear cells (PBMC) were separated and purified RNA was analyzed using Affymetrix GeneChip®. A fraction of PBMC was used for flow cytometry to define the cellular composition of the samples.

Results: 1882 differentially expressed genes that distinguished FHLH (n=22) from healthy controls (n=30) were identified (T-test, $p < 0.05$, Bonferroni correction). 623 differentially expressed genes that distinguished patients with FHLH2 from patients with genetic subtypes of FHLH that do not involve inactivating mutations in *PRF1*. We identified three genomic clusters that could distinguish all the nine patients with inactivating perforin gene mutations from thirteen patients who had unknown genetic cause of FHLH. The first cluster was comprised of genes involved in cell cycle regulation. The second cluster was enriched for genes involved in immune responses. The third cluster contained differentially expressed genes involved in mitochondrial function.

Conclusions: To gain insight into the genetic basis of the various forms of FHLH, we performed microarray analyses in 22 children. We identified genomic classifiers that clearly distinguish patients with perforin deficient form of hemophagocytic lymphohistiocytosis (FHLH2) from those who express normal levels of wild-type perforin but have another yet unknown genetic cause of the disease.

GENETIC PROFILES OF *MUNC13-4* IN PATIENTS WITH FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH) IN NORTH AMERICA.

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Introduction: Mutations in *PRF1*, *MUNC13-4* and *STX11* genes have been identified in patients with HLH worldwide. Here we describe the spectrum of mutations in *MUNC13-4* correlated with their clinical phenotypes for HLH patients in North America.

Materials and Methods: We investigated mutations in the *Munc13-4* gene from 215 unrelated patients with the clinical diagnosis of HLH. The entire coding region and exon/intron boundaries of *MUNC13-4* were analyzed by PCR and sequencing. Nature Killer (NK) cell function, perforin and granzyme B expression were also studied whenever is possible.

Summary of Results: Bi-allelic *MUNC13-4* mutations were found in 27 families (13%) and heterozygous *MUNC13-4* mutations were found in 39 patients (18%). Patients with bi-allelic mutations in *MUNC13-4* usually developed symptoms in the first year of life, and their NK function was markedly decreased or absent. The most striking finding in this study was the unusual high frequency of heterozygous mutations in *MUNC13-4* and the diverse associated phenotypes. In general, when the heterozygous mutation is a nonsense, frame shift or splice site mutation, patients tend to have early disease onset within the first year of life; however, if the heterozygous mutation is a missense mutation, patients tend to have a delayed disease onset in childhood or adulthood. Further investigation of *MUNC13-4* heterozygote is currently underway. One hypothesis is that there are mutations on the other allele that were not detected by the current test methodology; such as gross deletions that are not detected by PCR and sequencing, and mutations in the regulatory regions (e.g. promoter, enhancer and suppressor) that are not covered by the test design. A more daring hypothesis would explore the possibility that digenic/oligogenic effects influence the development of HLH in some patients, and also the possibility of dominant negative effects of *MUNC13-4* genotypes in these individuals. In this cohort of patients, we found total of 110 new sequence variants in the *MUNC13-4* gene, of which 18 are deleterious mutations, 47 are sequence variants of unknown significance, and 45 are benign polymorphisms. Some of these sequence variants only presented in certain ethnic groups, indicating possible founder effects. In addition, more than two dozen haplotypes were observed in this group of patients. Recent studies in other genes have shown that structural effects of certain SNP haplotypes can affect RNA processing and result in abnormal protein function; therefore, some of these haplotypes might contribute to the pathogenesis of HLH.

Conclusion: Both bi-allelic and single mutations in *MUNC13-4* were identified in patients with HLH in North America. These data expand our knowledge of the *MUNC13-4* genotypes in patients with HLH worldwide and support our recommendation that individuals with HLH syndromes in adulthood and/or secondary to other autoimmune conditions should be tested for mutations in *MUNC13-4*.

THERAPEUTIC RESULTS OF JLSG-02 PROTOCOL STUDY FOR MULTI-SYSTEM LANGERHANS CELL HISTIOCYTOSIS (LCH) PATIENTS: INTERIM REPORT.

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Background: Therapeutic outcome of multi-focal childhood Langerhans cell histiocytosis (LCH) has recently been improved with an introduction of prospective multi-institutional therapeutic trials. In our previous JLSG-96 protocol study conducted from 1996 to 2001, we attained a significantly low mortality rate (5.1%) in Japan. However, remained unsatisfactory were the low event free survival (<40%) and high reactivation (nearly 50%) rates. To further improve the quality of life in multi-focal LCH patients, we have started a modified treatment protocol (JLSG-02) from 2002. We analyzed the preliminary results of ongoing JLSG-02 protocol, compared with previous JLSG-96 results.

Patients and Methods: All patients were newly diagnosed children with multi-system LCH patients. JLSG-02 has been revised from JLSG-96 in; increase of prednisolone dosage at the induction phase and extension of total treatment duration from 7.5 months to 12 months for all patients and introduction of cyclosporine A (CSA) to the salvage regimen for progressive disease (PD) patients. Therapeutic results were compared for 92 patients treated with JLSG-02 protocol and 59 patients treated with JLSG-96 protocol.

Results: The 92 patients treated with JLSG-02 have been followed up for median 2.5 (range; 0.1-5.0) years. Response rate to the induction therapy at 6 weeks was not significant but slightly better in JLSG-02 than JLSG-96 results (79/92 (85.9%) vs. 45/59 (76.3%); $p=0.191$). Mortality rate was not different between the two protocol groups (4/92 (4.3%) vs. 3/59(5.1%); $p>0.999$). Three of the 5 patients who had PD during the induction therapy of JLSG-02 died even with the salvage therapy including CSA. No reactivation in a status of no evidence of LCH lesion with follow-up longer than 3 years was slightly better in JLSG-02 than JLSG-96 results (20/30(66.7%) vs. 17/38 (44.7%), $p=0.089$).

Conclusions: Although the results were still preliminary because only one third (30/92) of the patients were followed up for longer than 3 years in JLSG-02 protocol group, modification of treatment from previous protocol might have contributed to an improvement of initial response rate and reduction of reactivation. However, the fact that prognosis of patients with PD remained poor even with CSA assistance may require more refined therapy in these patients.

OUTCOME OF PATIENTS WITH LANGERHANS CELL HISTIOCYTOSIS TREATED WITH INDOMETHACIN.

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Background. Stephanie Munn et al (Med Pediatr Oncol 32: 247-249, 1999) concluded that indomethacin (IMT) was an useful therapy for Langerhans cell Histiocytosis (LCH) involving the bony skeleton and may have a role as first line treatment in single system bone disease.

Aims. To evaluate the response and outcome of LCH patients treated with IMT.

Methods. From February 1998 to May 2007, 12, 5 and 7 patients with symptomatic single system unifocal (SSUFBD), multifocal (SSMFBD) and multisystem disease (MSD) respectively were treated with IMT

Results. SSUFBD (12 patients): Median age was 3.7 years. The initial site of involvement was: skull 3 pts, femur 3, spine 2, pelvis 2 and scapula 1. One patient with temporal bone involvement (ear) who had complete response to LCH III HS protocol reactivated 3 months later, also received IMT. All patients were treated with IMT at the dose of 2 mg/kg/day. Moderate toxicity was found in 2 patients (somnolence). All the patients were evaluated at week 8: 7 had no evidence of active disease (NAD) and 5 were active disease better (ADB). The duration of treatment varied according the initial response from 2 to 12 months. Two patients still with stable disease after 5 and 6 months of IMT required treatment with prednisone to became NAD. Eleven patients are now without evidence of active disease and 1 is with ADB at the last evaluation. The median follow-up was 1.9 years and the survival free of reactivation was 8 months. SSMFBD (5 patients): Median age was 2.1 years. All patients were treated after reactivations with IMT (6 months-2 years) and 4 of them also received prednisone. Patients had at reactivation, bone involvement in 4 and vaginal mucosae involvement in one. Four patients responded to treatment and are NAD with a median FU of 1.25 years and a survival free of reactivation of 8 months. One patient with recurrent MFBD doesn't respond to IMT. MSD (7 patients): Median age was 1.75 years. All patients were treated after reactivation with IMT (6 months-2 years) and in 5 of them prednisone was given (one also received vinblastine, and another one, vincristine and cytarabine). All patients responded to initial treatment (NAD or ADB). Median FU was 2.75 years. Median survival free of reactivation was 9 months. One patient presented a mild and transitory increase of urea in blood. No reactivations were found during IMT treatment after NAD or ADB, in patients with SSUFBD, SSMFBD or MSD. The cost of Indomethacin in a patient of 15 kg per two months of treatment was 41 dollars.

Conclusions. IMT is an oral, inexpensive and almost non toxic drug. In this limited series, it may be a useful drug when used alone or with other medications for LCH pts with symptomatic unifocal bone disease, or after non risk organ reactivation in pts who had multifocal or multisystem disease at diagnosis.

FATIGUE IN ADULT PATIENTS WITH LANGERHANS CELL HISTIOCYTOSIS (LCH).

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Fatigue is present in many severe chronic diseases and was found in national population reports in 20-30% of adults. In most cases an underlying cause can be found. To answer the question to what extent fatigue is present in adult patients with LCH a questionnaire to all patients from the national adult LCH patients registry with proven diagnosis were sent.

The questionnaire was adopted from the Fatigue Coalition according to the ICD-10 criteria and contained 11 questions. Fatigue has to be assumed if more than 6 criteria were positive. The study was performed as a matched pair study. Matching was done for age and sex. At present 19 patients (mean age 44 ± 13 y) answered and could be evaluated. In the patient group 63% were female, 44% had single system lung disease and 34% multisystem disease.

In 74% of the LCH patients fatigue according to the criteria compared to the control group with 23%. Most frequent reported symptoms were sleeping disorders in 84%, followed by fatigue under exercise. Mean duration of fatigue was 4.8 ± 2.8 years. All patients with multisystem disease had fatigue whereas 80% of patients without fatigue had single system disease. No influence of chemotherapy or radiation therapy was found in patients with or without fatigue.

Fatigue is very frequent and long lasting in LCH patients especially in multisystem disease.

PET-CT IN PEDIATRIC LANGERHANS CELL HISTIOCYTOSIS.

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BACKGROUND – Assessment of disease extension and evaluation of response to therapy are major challenges in the management of LCH. Lesions develop in different organs, and patterns of response are organ-specific. In bones, lesions may remain stable by conventional imaging for prolonged periods of time despite intralesional response. Radiography and bone scanning with Tc99m-MDP may show normalization only with bone healing rather than demonstrating eradication of active lesions. Diagnostic tools that can evaluate lesional activity are thus important. We performed PET-CT in a group of patients with LCH in whom type, location, and number of lesions presented a challenge at diagnosis or follow-up.

PATIENTS AND METHODS – Six patients (7 months – 8 years) with multifocal or recurrent LCH underwent PET-CT. Three patients had multifocal bone and 3 had multisystem disease at the time of diagnosis. In two patients (1 multifocal and 1 multisystem), PET-CT was performed at diagnosis for better disease staging, and was subsequently used for response evaluation. In three patients (1 multifocal and 2 multisystem), PET-CT was used during follow-up to clarify the significance of abnormal radiographic findings. In one patient with multifocal bone, PET-CT was used at the time of disease reactivation to aid in the definition of disease sites and in the evaluation of response to therapy.

RESULTS – PET-CT detected more lesions than seen by conventional imaging methods at diagnosis or at the time of disease reactivation. In evaluation of response, FDG avidity correlated with response, whereas radiographs continued to show abnormalities. PET-CT was also useful in the interpretation of abnormal imaging findings during follow-up after therapy. Information provided by PET-CT was clinically useful in evaluating disease activity and response to therapy, and was more sensitive than conventional imaging methods.

CONCLUSIONS – PET-CT was found to be useful in the diagnosis and follow-up evaluation of patients with LCH, particularly those with residual lesions after therapy, and in the early detection of new lesions. The information provided by PET-CT may be useful in disease staging, development of new therapies, and monitoring of disease response.

**MACROPHAGE ACTIVATION SYNDROME OR
SECONDARY HEMOPHAGOCYTIC
LYMPHOHISTIOCYTOSIS: SUCCESSFUL MANAGEMENT
WITH HIGH DOSE METHYL PREDNISOLON THERAPY.**

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Background: Secondary hemophagocytic lymphohistiocytosis (HLH) is a serious complication of childhood systemic inflammatory disorders that is thought to be caused by excessive activation and proliferation of well differentiated macrophages. It occurs in a heterogeneous group of diseases, ranging from infections or neoplasms to hematological conditions and rheumatic disorders. Macrophage activation syndrome (MAS) is a term used by rheumatologists to describe a form of secondary HLH in association with rheumatic diseases in childhood. The management of this serious complication remains still a challenge.

Patients and study: Between January 2003-June 2007 a total of 12 patients (8 males/4 females, ages ranging from 1.5 years to 14 years) were diagnosed having MAS or secondary HLH. Eight patients had rheumatological diseases such as JRA (3pts), Kawasaki syndrome (1pt), SLE (1pt), Still disease (1pt), unidentified vasculitic syndrome (2pts). Two patients had ALL (one with polymyositis, one with active CMV infection), one patient had epilepsy and one patient had congenital heart disease with subacute bacterial endocarditis. At the time of secondary HLH diagnosis, all patients were febrile, bi or pancytopenic and nine of them had splenomegaly; they all had elevated triglyceride and ferritin levels; hemophagocytosis could be demonstrated in all patients in the bone marrow aspirates. Five patients had renal failure, eleven patients elevated liver enzymes. All patients except one were followed in the pediatric ICU because of coagulopathy and/or multiorgan failure and/or circulatory collapse. The first two patients, both with renal failure, treated according to the HLH-94 protocol and were lost despite aggressive supportive treatment. Remaining ten patients were treated mainly with high dose methylprednisolon (30 mg/kg/dx3 days, 20 mg/kg/dx2 days, 10mg/kg/dx1 day, 5 mg/kg/dx1 day and then 2 mg/kg/d for 3 weeks and then steroids were tapered gradually). The treatment of the underlying disease was not discontinued. In all patients complete resolution of the symptoms was achieved. Eight patients did not experience relapses after cessation of steroid therapy. The patient with ALL+CMV infection relapsed three times with different triggering agents but successfully treated with steroids. As tapering of the steroids was not possible, substitution with cyclosporine A was done.

Conclusion: MAS and secondary HLH are life threatening severe conditions. Although the appropriate treatment remains to be defined high dose methylprednisolon seems to be feasible treatment strategy in these subset of patients.

**ANTITHYMOCYTE GLOBULINS BASED IMMUNOTHERAPY
(ABI) OF FAMILIAL HEMOPHAGOCYTIC
LYMPHOHISTIOCYTOSIS:
A SINGLE CENTER RETROSPECTIVE
REPORT OF 38 PATIENTS.**

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Objectives: Familial hemophagocytic lymphohistiocytosis (FHLH) is a genetically determined condition, characterized by unremitting CD8 T lymphocyte and macrophage activation that leads to death in the absence of therapy. Based on the immunological pathophysiology of FHLH, we propose a therapy with an antithymocyte globulins based immunotherapy (ABI) using a combination of anti thymocyte globulins (ATG) with corticosteroids, cyclosporine A and intrathecal injections of methotrexate.

Methods: We retrospectively analyzed the outcome of ATG-based therapy that was performed in 38 consecutive patients who had FHLH and were treated in a single center between 1991 and 2005. Overall, they received 45 courses of ATG (5 to 10 mg/kg/day x 5).

Results: *Tolerance* : this regimen was associated with infections following 10 of 45 courses of ATG. There were six events after 11 ATG courses given as second-line therapy against four after 34 ATG courses in patients primarily treated with ATG ($p < 0.01$). *Efficacy:* ATG administration led to rapid and complete response (CR) of FHLH in 73% of cases, partial response (PR) in 24% with no response noted only once. When hematopoietic stem cell transplantation (HSCT) was performed early after CR or PR induction, it led to a high rate of cure, in 16 of 19 cases. Overall survival is 21 out of 38 with 4 toxic deaths.

Conclusion: As based on this retrospective analysis, ATG based immunotherapy (ABI) of FHLH is efficient and carries an acceptable toxicity when used as a first treatment of FHLH. It would be worthwhile performing a prospective study comparing the ATG based immunotherapy (ABI) to the "reference" HLH-2004 protocol. For the future, further improvements could relate to more targeted immuno-suppression aimed at further reducing toxicity.

EXPANSION OF REGULATORY T CELLS IN PATIENTS WITH LANGERHANS CELL HISTIOCYTOSIS.

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Background. The pathophysiology of LCH is unclear, but the uncontrolled proliferation of LC is believed to be the primary event in the formation of granulomas.

Methods and patients: Paraffin-embedded (n=9) or frozen (n=12) or fresh biopsies (n=3) from eosinophilic granulomas, or peripheral blood specimens (n=25) from 40 pediatric patients with LCH were obtained after written witnessed informed consent was obtained from the parents. Proliferating cells were identified with a double staining for Ki67 and cellular Ag on tissue sections. IL-10 transcripts were quantified by real time PCR in frozen biopsies. Rank and RankL were detected by immunohistochemistry (IHC) on paraffin-embedded sections. T-regs were identified in biopsies by immunofluorescence and IHC. The frequency of T-regs was evaluated in fresh biopsies and in the peripheral blood by FACS. Tuberculin PPD-skin reaction were performed in 7 patients previously vaccinated with BCG.

Principal Findings

In the present study we found that the proliferating index of LC was low (~1.9 %) and we did not observed expansion of a monocyte or DC compartment in patients. We found that LCH lesions were a site of active inflammation, tissue remodeling, and neo-angiogenesis, the majority of proliferating cells being endothelial cells, fibroblasts and polyclonal T lymphocytes. Within granuloma, IL10 was abundant, LC expressed Rank, and CD4+ CD25^{high} FoxP3^{high} regulatory T cells (Tregs) represented 20% of T cells, and were found in close contact with LC. FoxP3⁺ Tregs were also expanded, in comparison with controls, in the blood of LCH patients with active disease, among whom 7 out of 7 tested exhibited impaired skin delayed-type hypersensitivity (DTH) response. In contrast, blood Tregs number were found to be normal after remission of LCH.

Conclusions/Significance

These findings indicate that LC accumulation in LCH results from survival rather than uncontrolled proliferation, and is associated with the expansion of regulatory T cells. These data suggest that LC may be involved in the expansion of Tregs in vivo, resulting in the failure of the host immune system to eliminate LCH cells. Thus Tregs could be a therapeutic target in LCH.

THE TIMING OF PITUITARY INVOLVEMENT IN LANGERHANS CELL HISTIOCYTOSIS CORRELATES WITH THE DISEASE EXTENSION.

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Aim: To describe the chronology of pituitary and extra-pituitary involvements in langerhans cell histiocytosis (LCH) and its correlation with disease extension.

Patients and Methods : Among 952 patients with LCH enrolled in the French data base until may 2007, 179 patients had presented a diabetes insipidus. The time of DI was the time of the medical test. If the delay between the involvement of two distinct organs (or two distinct bones) is below 3 months, the two involvements are considered to belong to the same occurrence of the disease, otherwise each involvement constitutes a distinct occurrence of the disease. The DI-pituitary involvement was considered as inaugural if it was the only organ involved at the first occurrence of the disease, concomitant if it was present at the first occurrence of the disease in association of any other organ and secondary if it was observed in any subsequent occurrences of the disease. Neurodegenerative LCH was considered if neurological symptoms were associated with a typical brain MRI. Brain MRI abnormalities, without clinical symptom was considered as MRI-degenerative LCH.

Preliminary results: Patients with inaugural DI ("LCH with "primitive pituitary involvement") (n=29) had a different clinical presentation and outcome compare to concomitant (n=50) or secondary DI (n=100). They are older at LCH diagnosis (9.7 y vs 3.2 y and 2.2 y respectively) than concomitant and secondary DI. Organ's involvement, during all the observation time (median time: 9.1 y, 6.1 y and 12.3 years respectively) demonstrates differences in the extent of the disease. Inaugural DI had statistically less skin (27%; 48%, 63% respectively), node (0, 20%, 20% respectively), bone (44%, 88%, 91% respectively), ENT (24% 44%, 54% respectively) involvements. Thyroid gland involvement (10%, 0, 2% respectively), and hypothalamic tumor (28%, 6%, 4% respectively), GH deficiency (59%, 44%, 31% respectively) were statistically more frequent. Lung involvement (35%, 18%, 20%) and neurodegenerative syndrome (17%, 8% 9% respectively) were, not statistically, more frequent. Overall the disease course showed fewer occurrences in inaugural DI vs concomitant or secondary DI (2.8, 2.3 and 4.4 respectively).

Conclusion: Primitive LCH of the pituitary have a distinct outcome and natural history. The timing of the onset of DI correlates with the natural history of LCH (maximal extent, number of occurrences, permanent consequences).

NEURODEGENERATIVE CENTRAL NERVOUS SYSTEM DISEASE AS LATE SEQUELAE OF LANGERHANS CELL HISTIOCYTOSIS; JLSG REPORT.

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Purpose: The long-term outcome of neurodegenerative CNS disease of Langerhans cell histiocytosis (ND-CNS-LCH) patients is dismal. Multi-institutional case study is essential to understand the natural history of ND-CNS-LCH disease and to find out useful prophylactic and therapeutic measures.

Patients and Methods: We analyzed clinical characteristics of 11 patients (10 males, 1 female) with ND-CNS-LCH disease registered in the Japan LCH Study Group (JLSG), which consisted of 5 patients in whom abnormal MRIs were noted prior to neurological symptoms (Group 1; G1) and of 6 patients with neurological symptoms detected first (Group 2; G2). Four patients were treated with an intravenous immune globulin (IVIG)-containing regimen for longer than 12 months, while seven were observed without any specific treatment. The EDSS scores were employed to evaluate the grade and progression of ND-CNS-LCH disease.

Results: All 11 patients had multi-focal (MM-type) LCH; in the year 1985-87 (n=2) and after the year 1996 (n=9). Nine patients developed LCH at the ages of 1-2 yrs and two at the ages of 4.8 and 16 yrs, respectively. ND-CNS-LCH disease developed after an interval of median 3.9 (range; 0-10) years from initial diagnosis. ND-CNS-LCH was diagnosed at younger ages (2.5 to 5.5 yrs) in the G1 group and at older ages (5 to 23 yrs) in the G2 group patients. Increases in the EDSS scores (calculated as EDSS score increase/ year) were delayed in the IVIG-treated patients, compared to the non-IVIG patients (0.27/year vs. 0.49/year). At the last follow-up, four patients maintained EDSS scores <3, five patients had scores 3-5 and two patients had scores >7.0. One patient died of unknown causes at age 18 yrs. We also estimated the incidence of ND-CNS-LCH in Japan as 5.3% of MM-type of pediatric (age <15yrs) LCH patients.

Conclusions: This study demonstrates the importance of early detection of ND-CNS-LCH by brain MRI, particularly in the follow-up of male patients who developed MM-type LCH in early infancy. Since the progression of the disease with time is evident, application of effective therapeutic measures during the asymptomatic phase of the disease is essential.

CSF MARKERS OF NEURODEGENERATION IN CHILDREN WITH LCH .

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Introduction: The incidence of neurodegeneration in LCH has shown to be higher than previously appreciated, and has been suggested to affect at least 20-25% of all LCH patients. Since the development of CNS disease in LCH is slow, it is important to detect neurodegeneration early. Moreover, it is particularly important to be able to assess the degree of the ongoing neurodegenerative process to be able to evaluate the potential efficacy of therapeutic interventions. We, therefore, studied biomarkers of neurodegeneration in the cerebrospinal fluid (CSF) of patients with neurodegenerative LCH and correlated these with clinical and neuroradiological findings to evaluate their relevance as markers of neurodegeneration in LCH.

Material and Methods: We studied nine Swedish patients diagnosed with LCH at 0.4-6.3 years of age (median 2.1), who had endocrine deficiencies, neuromotor, cognitive or/and behavioural abnormalities, and in addition magnetic resonance images (MRI) findings of LCH neurodegeneration. The CSF samples were drawn 4.6-12.8 years after LCH diagnosis. In two patients CSF was analyzed longitudinally, during a period of 14-19 months. The CSF biomarkers analyzed were neurofilament protein light chain (NF-L), glial fibrillary acid protein (GFAP), and total tau protein (TAU). As controls served 110 children with newly diagnosed acute lymphoblastic leukemia (ALL) without known CNS involvement.

Results: In 8/9 patients the CSF concentration of GFAP was abnormal at the first lumbar puncture (LP), four patients had abnormal NF-L levels, and TAU was elevated in six patients. We then investigated the association between the biomarkers and the clinical and neuroradiological observations. Two patients with severe CNS-LCH both had elevated levels of all three neurodegenerative markers. The one patient with the most obvious clinical and radiological signs of progressive LCH-CNS disease also showed the highest levels of NF-L and GFAP. Her TAU-levels were also elevated. On the contrary, three patients with non-active disease and resolution at the time of evaluation had low TAU levels and normal or only slightly elevated NF-L. Moreover, the TAU levels in the CSF correlated with the systemic disease activity ($\rho=0.66$, $p=0.03$), as well as with the disease activity progression/regression ($\rho=0.66$, $p=0.03$). Finally, NF-L and TAU tended to be positively correlated ($R=0.58$, $p=0.08$). Notably, we experienced more frequent severe complications during LP in LCH patients than in ALL patients.

Conclusions: Our findings indicate that the biomarkers NF-L and TAU, and possibly also GFAP, in CSF can be used to evaluate ongoing neurodegeneration in LCH. Preliminary, it appears as if elevated NF-L levels are particularly alarming. Our findings may be of a value diagnostically, as well as for the evaluation of therapeutic interventions.

**EVALUATION OF THE [¹⁸F]FDG PET PATTERN
IN DEGENERATIVE-LIKE
NEURO-LANGERHANS CELL HISTIOCYTOSIS.**

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Objectives: Degenerative-like neuro-Langerhans cell histiocytosis (DLN-LCH) is a rare complication of LCH, marked by progressive cerebellar ataxia associated with spastic tetraparesis and cognitive disorders. Magnetic Resonance Imaging (MRI) is the gold standard to investigate central nervous system (CNS) lesions of DLN-LCH but few is known about functional changes observed in the disease. In order to evaluate CNS metabolic changes a group of patients with DLN-LCH having clinical signs of central nervous system involvement was studied using positron emission tomography (PET) and fluorodeoxyglucose labelled with fluorine 18 ([¹⁸F]-FDG).

Methods: Seven patients presenting clinical and neuroradiological CNS involvement of DLN-LCH were enrolled in this study (4 male and 3 females; mean age 26 years). Brain MRI was obtained for all subjects using a 1.5T imager. PET studies were performed with an ECAT EXACT HR+ scanner using [¹⁸F]-FDG. The PET scans were compared to 21 normal subjects (12 men and 9 women, mean age 35 years). The groups were compared using statistical parametric mapping (SPM99) and a voxel-by-voxel *t* statistic analysis. Differences were considered significant at the *p*<0.001 level.

Results: MRI abnormalities were observed for all the patients: 6/7 presented a cerebellar atrophy; 4/7 presented dentate nuclei changes with or without globus pallidus abnormalities. An individual visual imaging analysis showed a decrease uptake of [¹⁸F]-FDG in vermis, caudate nuclei and cortical cortex observed in 6, 6 and 5 patients respectively. Increased uptake of [¹⁸F]-FDG was observed in amygdala body of 4 patients presenting the shortest evolution of the neurological disease. The SPM analysis revealed significant [¹⁸F]-FDG uptake abnormalities (*p*<0.001) corresponding to bilateral hypometabolism in cortical cortex, basal ganglia and vermis and a bilateral hypermetabolism in amygdala body.

Conclusions: This study showed a recurrent CNS metabolic profile in patients with DLN-LCH suggesting a striatal involvement in the pathophysiology of this disease. Since, neurological symptoms may be the presenting symptom of LCH, PET with [¹⁸F]-FDG may be helpful to distinguish DLN-LCH from other causes of progressive spinocerebellar ataxia.

**VALUE OF BRAIN STEM EVOKED ACOUSTIC
POTENTIALS IN THE ASSESSMENT OF
NEURODEGENERATIVE CNS LCH.**

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Introduction: Neurodegenerative lesions in the cerebellum, pons and basal ganglia on MRI (radiological ND=RND) are diagnosed in an increasing number of LCH patients. In a recent follow up study, almost 70% of the patients with RND showed progression of MRI lesions over about 3 years. In 25% neuropsychological symptoms such as gait disturbances, ataxia, reflex abnormalities, tremor and behavioural disturbances (clinical ND) were reported. The course of MRI and clinical findings assessed by neurological and psychological tests did not correlate in all patients. More sensitive methods are needed to early detect symptoms, to support therapeutic decisions and to monitor the treatment effect.

Materials and Methods: Brain stem evoked acoustic potentials (BAEP) are generated in the brain stem, increased latencies reflect transmission problems of the acoustic stimulus in the auditory pathway corresponding to decay of nerve cells. 17 patients with RND were assessed with BAEP. They were 5 females, 12 males. RND was diagnosed 4 years in median (range 0.2 – 17 yrs) after LCH diagnosis. BAEPs of all patients were reviewed by an experienced paediatric neurologist (RS).

Results: Normal BAEP latencies were seen in 5 patients. Increased latencies were measured in 12 patients. 7/17 patients had clinical ND; 6 of them showed increased latencies in the central auditory pathway in median 2 years (2m – 10 yrs) after diagnosis of RND and median 6.5 years (range 3 – 26 yrs) after the diagnosis of LCH.

10/17 patients had no evidence of clinical ND. 6 of them had increased latencies in the central auditory pathway in median 2 years (3 yrs – 8 yrs) after the diagnosis of RND and median 2 years (0.9 – 6.5 yrs) after the diagnosis of LCH.

16/17 patients had ≥ 2 follow-up-MRI studies after diagnosis of RND. 11/16 showed progressive MRI changes over a median observation time of 7 years (8m – 11yrs). In 5 patients MRI lesions remained stable over 4 years in median (range 2 – 4.2 years).

In 5/17 patients follow-up-BAEP-exams were evaluable over median 5 years (1 – 6.5 yrs). 2 patients without clinical ND upon neuropsychological testing and stable MRI findings had stable normal BAEP-results over 5 and 6 years.

3 patients showed a progression of BAEP-latencies over 1, 3 and 6 years: In 2 patients progression of BAEP-latencies correlated with MRI deterioration, one of these developed clinical ND after BAEP deterioration. In the other patients BAEP deterioration was the first and preclinical sign of functional nerve cell loss.

Conclusion: Based on these preliminary data BAEPs appear to be a suitable non-invasive method to assess and follow neurodegenerative CNS-LCH and seem to be more sensitive than clinical neuropsychological testing.