

### Autoimmunity associated with Erdheim-Chester disease improves with BRAF/MEK inhibitors

Erdheim-Chester disease (ECD) is a rare non-Langerhans cell histiocytosis characterized by the infiltration of various tissues by CD68-positive (+), CD1a-negative (-) spumous histiocytes. Since the discovery that the RAS-RAF-MEK-ERK pathway is altered in almost all cases of ECD, it is now considered to be a myeloid neoplasm. Indeed, 67% of ECD patients harbor the somatic *BRAF*<sup>V600E</sup> mutation causing the constitutive activation of the BRAF-MEK pathway.<sup>1</sup> However, the mechanisms behind the accumulation of histiocytes in ECD are not fully understood. ECD lesions typically include inflammatory cells (mainly lymphocytes) in addition to pathological histiocytes. Langerhans-cell histiocytosis (LCH) is another L-group histiocytosis, which is also considered to be a clonal disease.<sup>2</sup> However, typical LCH lesions are inflammatory with abundant CD1a<sup>+</sup> histiocytes. LCH more commonly affects children, while ECD affects adults, typically after 50 years old. ECD and LCH may be associated in so-called “mixed histiocytosis”, which supports the hypothesis of a common cell of origin in these two diseases and encourages the inclusion of both of them in the L-group of the 2016 revised classification of histiocytoses.<sup>3</sup>

Histiocytes, which encompass monocytes, macrophages, and dendritic cells (DC), are heterogeneous cells that play an important role in the physiology, initiation, and resolution of inflammation. Macrophages and DC are crucial for the regulation of the immune system. Autoimmunity is present in a broad spectrum of diseases in which histiocytes are pathologically altered, ranging from metabolic diseases to histiocytic disorders. Autoimmunity has also been described in myeloid neoplasms.<sup>4</sup> In Gaucher diseases, antinuclear antibodies (ANA) are found in up to 25% of cases.<sup>5</sup> In Destombes-Rosai-Dorfman disease (DRD), another rare non-Langerhans-cell histiocytosis belonging to the R-group of histiocytoses, autoimmunity has been reported in up to 20% of cases.<sup>6</sup> There are several reports of DRD associated with systemic lupus erythematosus (SLE),<sup>7</sup> membranous glomerulopathy, or autoimmune cytopenia. In contrast to DRD, autoimmunity has not been evaluated in L-group histiocytoses. Few cases of LCH or ECD associated with autoimmune disease (AID) have been previously reported.<sup>8</sup> Recently, a case of polymyalgia rheumat-

ica, an immune disease of unknown cause, was successfully treated with cobimetinib, an MEK inhibitor.<sup>9</sup> BRAF and MEK inhibitors are widely used in L-group histiocytoses since they specifically target the RAS-RAF-MEK-ERK pathway.<sup>10</sup> We aimed to determine the prevalence of AID and biological autoimmunity in L-group histiocytoses, and to evaluate the impact of BRAF and MEK inhibitors on the clinical and biological immune manifestations of histiocytoses.

We screened all patients with L-group histiocytoses (ECD and LCH) seen in the French National Reference Center of Histiocytoses of the Pitié-Salpêtrière Hospital, Paris. All patients had a clinical and radiological presentation compatible with L-group histiocytosis, and tissue samples were centrally reviewed for pathological confirmation. The presence of the *BRAF*<sup>V600E</sup> mutation was searched as previously described.<sup>11</sup> The study was conducted according to the French data protection authority (CNIL) methodology reference and in accordance with the Declaration of Helsinki. Demographic, clinical, biological, imaging data, and treatments were recorded from medical charts. Patients were included if they had at least one determination of ANA [HEP-2000 slides (Immunoconcepts, Sacramento, CA, USA) with HEP-2000™ cells]. A test >1/80 was considered positive. When ANA test was positive, anti-double stranded DNA antibodies were identified with ELISA tests (ETI-dsDNA, Diasorin, Italy), and anti-extractable nuclear antibodies (ENA) were screened with ELISA (ANA screen, ORGENTEC, France), then confirmed with specific multiplex (Luminex) testing (FIDIS Connective, THERADIAG, France). Additionally, patients were all checked for the presence of other antibodies, such as anticardiolipid (ACL) antibodies, antibeta2 glycoprotein 1 (antiβ2GP1), and lupus anticoagulant (LA), in accordance with international guidelines. Antiphospholipid antibodies (APL) designed ACL antibodies, antiβ2GP1 antibodies, and/or LA. For APL antibodies, two successive determinations, separated by at least 12 weeks, were required to consider the patient as “positive”. We also systematically checked for the presence of AID among a list of 22 diseases (*Online Supplementary Table S1*). The criteria for AID were verified from medical charts and/or biological analyses, and were derived from the work of international groups. Patients were treated according to routine care. The presence of autoimmunity refers in the text to either clinical (AID) and/or biological evidence of autoimmunity. Targeted therapy, corresponding to BRAF inhibitors

**Table 1.** Patients of L-group histiocytosis with autoimmunity.

	ECD (n=167)	Mixed histiocytosis (n=29)	LCH (n=35)	P*	P**
Age at histiocytosis diagnosis, mean (SD)	57 (15)	58 (12)	36 (15)	<0.0001	<0.0001
Age at ANA determination, mean (SD)	61 (15)	62 (11)	44 (16)	<0.0001	<0.0001
Sex (M/F)	123/44	15/14	16/19	0.006	0.001
AID without ANA/APL (group 1)	16 (10%)	0 (0%)	1 (3%)	0.091	0.084
AID with ANA/APL (group 2)	6 (4%)	1 (3%)	0 (0%)		
ANA/APL/Autoantibodies without defined AID (group 3)	47 (28%)	12 (41%)	6 (17%)		
Total of patients with autoimmunity	69 (41%)	13 (45%)	7 (20%)	0.015	0.047

AID: autoimmune disease; ANA: antinuclear antibodies; APL: antiphospholipid antibodies; APS: antiphospholipid syndrome; ECD: Erdheim Chester disease; F: female; LCH: Langerhans cell histiocytosis; n: number; M: male; SD: Standard Deviation; SGS: Sjögren syndrome; SLE: systemic lupus erythematosus. Patients with autoimmunity were classified into three groups depending on their clinical and biological immune profile: Group 1: presence of SLE, SGS, APS, or other AID without ANA and/or persistent APL; Group 2: presence of AID (except SLE, SGS and APS) with ANA and/or persistent APL; Group 3: presence of ANA, persistent APL, or other autoantibodies, without criteria for AID. \*Comparison between ECD (including mixed histiocytosis) and LCH. \*\*Comparison between three groups: AID, ANA, and APL.

(vemurafenib and dabrafenib) and MEK inhibitors (cobimetinib and trametinib) were prescribed according to physicians' decisions, usually when the severity of the disease required. For patients treated with BRAF or MEK inhibitors, we determined the outcomes of AID and biological autoimmune parameters and noted the results of successive determinations of antiphospholipid titers. Follow up ended in August 2018. Comparisons were performed with Student *t*-test, ANOVA, the Kruskal-Wallis

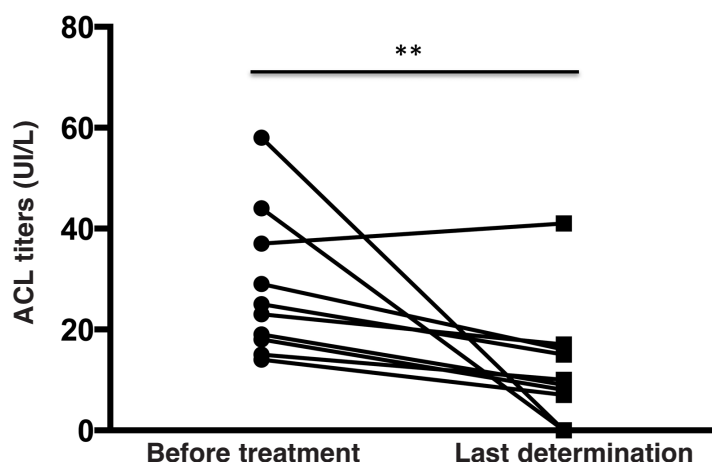
test, Wilcoxon matched pairs signed-rank test, Mann-Whitney U test, or Fisher exact test when appropriate. Survival curves were built using the Kaplan-Meier method considering the time of diagnosis of ECD to death or last follow up. All tests were two-sided;  $P < 0.05$  was considered statistically significant. Statistical analyses were performed using the GraphPad Prism V 6.0 (GraphPad software, La Jolla, CA, USA).

We screened 276 patients: 187 patients with ECD, 51

**Table 2.** Demographic, clinical, and biological characteristics of Erdheim-Chester disease patients with and without autoimmunity.

	All (n=196)	Autoimmunity (n=82)	No autoimmunity (n=114)	P
Sex (M/F)	138/58	53/29	85/29	0.15
Age at diagnosis (mean, SD)	57 (14)	58 (14)	57 (15)	0.36
Age at ANA dosage (mean, SD)	61 (14)	62 (12)	60 (15)	0.30
BRAF status, n (%)				
V600E	108/166 (65%)	43/71 (61%)	65/95 (68%)	0.32
Mixed histiocytosis, n (%)	29 (15%)	13 (16%)	16 (14%)	0.84
ECD involvements, n (%)				
Cardiac involvement	96 (49%)	42 (51%)	54 (47%)	0.66
Vascular involvement	116 (59%)	49 (60%)	67 (59%)	1.00
Xanthelasma	45 (23%)	21 (26%)	24 (21%)	0.49
Diabetes insipidus	51 (26%)	22 (27%)	29 (25%)	0.87
CNS involvement	69 (35%)	26 (32%)	43 (38%)	0.45
Retro-orbital involvement	38 (19%)	18 (22%)	20 (18%)	0.47
Retroperitoneal involvement	122 (62%)	47 (57%)	75 (66%)	0.24
Deaths, n (%)	41 (21%)	21 (26%)	20 (18%)	0.21
Treatments, n (%)				
Corticosteroids	58 (30%)	21 (26%)	37 (32%)	0.34
IFN- $\alpha$ or PEG-IFN- $\alpha$	145 (74%)	63 (77%)	82 (72%)	0.51
Anakinra	28 (14%)	9 (11%)	19 (17%)	0.31
Infliximab	19 (10%)	8 (10%)	11 (10%)	1.00
Targeted therapy, n (%)	75 (38%)	32 (39%)	43 (38%)	0.88
Vemurafenib	66 (34%)	28 (34%)	38 (33%)	–
Dabrafenib	9 (5%)	4 (5%)	5 (4%)	–
Cobimetinib	29 (15%)	11 (13%)	18 (16%)	–
Autoimmune disease, n (%)	23 (12%)	23 (28%)	0	
Autoimmune thyroiditis	8 (4%)	8 (10%)	0	
Systemic lupus erythematosus	3 (1.5%)	3 (4%)	0	–
Primary Sjogren syndrome	3 (1.5%)	3 (4%)	0	
Autoimmune biology, n (%)				
ANA > 1/80	50 (26%)	50 (61%)	0	
AntidsDNA +	10 (5%)	10 (12%)	0	
AntiSS-A +	9 (5%)	9 (11%)	0	
AntiSS-B +	1 (0.5%)	1 (1%)	0	
APL biology*	36 (18%)	36 (44%)	0	
LA	5 (3%)	5 (6%)	0	
Anticardiolipid +	31 (16%)	31 (38%)	0	
Anti $\beta$ 2GPI +	7 (4%)	7 (9%)	0	

M: male; F: female; IFN- $\alpha$ : interferon- $\alpha$ ; ANA: antinuclear antibodies; antidsDNA: antidouble stranded deoxyribonucleic acid antibodies; ECD: Erdheim-Chester disease; CNS: central nervous system; SD: Standard Deviation; APL biology: antiphospholipid biology; LA: lupus anticoagulant; ACL: anticardiolipid antibodies; anti $\beta$ 2GPI: antibody 2 glycoprotein 1 antibodies; n: number. \*Refers to LA, ACL and/or anti $\beta$ 2GPI positivity.



**Figure 1.** Anticardiolipin antibody titers in Erdheim-Chester disease (ECD) patients treated with targeted therapies (BRAF and/or MEK inhibitors). Among 75 ECD patients treated with BRAF and/or MEK inhibitors, 18 had persistent anticardiolipin (ACL) antibodies. Among them, we studied the evolution of ACL titers in 11. "Before treatment": the last value of the ACL IgG or IgM level in the six months preceding the initiation of targeted therapy; "Last determination": the last value of the ACL IgG or IgM level while under targeted therapy. ACL titers significantly decreased between baseline and the last determination ( $P=0.0049$ ).

with LCH, and 38 with mixed histiocytosis. Among them, 45 were excluded: 30 because of insufficient data in medical charts; 15 because they had no determination of autoimmune biology (*Online Supplementary Figure S1*). Finally, 231 patients were included in the analysis, corresponding to 167 with ECD, 35 with LCH, and 29 with mixed histiocytosis. The global prevalence of autoimmunity (biological and/or clinical) was 41% in ECD, 45% in mixed histiocytosis, and 20% in LCH (Table 1). The prevalence of autoimmunity was higher in ECD than in LCH (42% vs. 20%;  $P=0.015$ ). The prevalence of AID was also higher in ECD than in LCH, although this difference did not reach statistical significance (12% vs. 3%;  $P=0.14$ ). Among patients with ECD, there was no difference in clinical characteristics or treatments between patients with and without autoimmunity (Table 2). The *BRAF* status was similar in patients with and without autoimmunity. There was no significant difference in survival between patients with and patients without autoimmunity. The median survival was 127 months in patients with autoimmunity (undefined in patients without autoimmunity [Hazard Ratio 1.63, (0.86-3.12);  $P=0.13$ ] (*Online Supplementary Figure S2*). Twenty-three patients out of 196 (12%) had AID associated with ECD. These patients were 14 males and nine females, with a median age at ECD diagnosis of 57 years (range: 26-86 years). The AID cases included autoimmune thyroiditis ( $n=8$ ), primary Sjögren syndrome ( $n=3$ ), SLE ( $n=3$ ), pernicious anemia ( $n=2$ ), polymyalgia rheumatica ( $n=2$ ), APS ( $n=1$ ), type 1 diabetes ( $n=2$ ), coeliac disease ( $n=1$ ), autoimmune hemolytic anemia ( $n=1$ ), immune thrombocytopenic purpura ( $n=1$ ), and autoimmune alveolitis ( $n=1$ ). Two patients had an association of two AID: thyroiditis and pernicious anemia in one patient, coeliac disease and SLE in another.

Autoimmune disease was present before the diagnosis of ECD in 12 cases. Among them, the median time between AID and ECD diagnosis was 84 months (range: 0-336 months). The AID occurred during or after the ECD diagnosis in 11 cases. Overall, 145 patients (74%) with ECD were treated with interferon- $\alpha$ , and 19 (10%) were treated with infliximab. Sixty-three patients (43%) among those who received interferon- $\alpha$  had autoimmunity, whereas 19 (37%) among those who did not receive interferon- $\alpha$  had autoimmunity ( $P=0.51$ ). Eight patients (42%) had autoimmunity among those who received infliximab, and 74 patients (42%) had autoimmunity

among those who did not ( $P=1.00$ ). Among patients with AID and ECD treated with interferon- $\alpha$ , one presented a SLE flare with multiple arthritis during the treatment, which was therefore stopped. Other patients did not experience a worsening of AID under this treatment.

Among ECD patients, 75 were treated with targeted therapy (BRAF and/or MEK inhibitor). Among patients treated with targeted therapy, six patients (8%) had AID, 18 (24%) had positive ANA, and 18 (24%) had persistent antiphospholipid antibodies, including two with LA, 18 with ACL, and two with anti $\beta$ 2GPI antibodies. The ACL antibody titers significantly decreased during treatment ( $P=0.0049$ ) (Figure 1). One patient with SLE did not have any flares during targeted therapy (17 months of follow up), whereas he experienced one flare during the six months preceding targeted therapy.

L-group histiocytoses refer to myeloproliferative neoplasms, caused by the constitutive activation of the RAS-RAF-MEK-ERK pathway. This leads to the qualitative and quantitative modification of DC, monocytes, and macrophages. It is possible to infer from our results that pathological histiocytes show altered functions of immune homeostasis through the modification of their phagocytosis or antigen presentation functions. Several studies demonstrated a cytokine/chemokine network in ECD lesions that may contribute to the recruitment and activation of pathological histiocytes. In ECD lesions, inflammatory cells are also present, such as eosinophil polynucleated cells and lymphocytes. Tumor necrosis factor (TNF)- $\alpha$  and TNF-receptor are increased in ECD patients compared to controls, demonstrating that TNF- $\alpha$  is an important regulator of inflammation in ECD.<sup>12</sup> It is not clear if the modifications of these cytokines act as a cause or a consequence of the pathological changes of histiocytes. The microenvironment around pathological histiocytes, with the recruitment of immune cells, can participate in the induction of autoimmunity. An alteration of the destruction of circulating DNA by pathological histiocytes can also increase ANA occurrence. Fas ligand (FasL), an apoptosis-inducing member of the TNF cytokine family, and its receptor Fas are critical for the control of chronic immune responses and the prevention of autoimmunity. FasL (Deltam/Deltam) mice, in which T cells lack membrane-bound FasL, succumb to SLE-like autoimmune disease and histiocytic sarcoma.<sup>13</sup> Hereditary Fas ligand deficits have been associated with DRD but not with L-group

histiocytosis. However, since in our study patients were not investigated for Fas dosages, we cannot exclude the possibility that an acquired or constitutional deficit could explain the association between ECD and autoimmunity. ECD patients with severe manifestations are frequently treated with BRAF or MEK inhibitors.<sup>14</sup> Recent evidence suggests that the MEK inhibitor could be used in autoimmune or inflammatory diseases.<sup>9</sup> The RAS kinase has two downstream effectors: the RAF-MEK-ERK and the PI3KC-AKT-mTOR pathways. ERK expression is increased in several immune diseases.<sup>15,16</sup> In our study, we showed that ACL titers significantly decreased under BRAF or MEK inhibitors. We cannot draw any conclusions about a specific effect of the MAPK pathway inhibition, or an anti-inflammatory effect on histiocytes. Interestingly, mTOR inhibitors have been proposed for ECD treatment, and were also shown efficacious in antiphospholipid syndrome.<sup>17,18</sup> mTOR inhibitors could probably be a good choice as a first-line treatment in patients with ECD without severe manifestation and AID when interferon- $\alpha$  cannot be used. In this study, it was more difficult to draw conclusions concerning the outcomes of AID during targeted therapy or interferon- $\alpha$  due to the heterogeneity of the diseases, the short duration of targeted treatments, and the absence of a control group. Interferon- $\alpha$  can probably be safely used in patients with thyroiditis, pernicious anemia, or type 1 diabetes, but should only be used with caution in patients with SLE, as we observed a flare in one ECD patient of the cohort under this treatment.

Interestingly, IgG4-related disease has been described in association with histiocytic disorders.<sup>19</sup> However, we did not observe this association in our cohort of patients.

This study has several limitations. First, it is a retrospective study and we can expect that some cases of AID were not mentioned in medical charts. This bias was partially controlled because the medical charts were systematically checked for a list of AID cases. However, some type 1 diabetes cases may have been misdiagnosed as type 2, or some patients may not have mentioned a past history of AID. Another bias may be the prescription of interferon- $\alpha$  and infliximab. However, most patients received a diagnosis of AID before the prescription of such medications, and there was no difference in the proportion of patients having received these drugs between the groups with and those without autoimmunity.

In conclusion, we report a high prevalence of autoimmunity (both AID and autoimmune biology) in ECD. ACL titers significantly decreased when patients were placed under targeted therapies. These results may help to identify new therapeutic approaches in autoimmune diseases and to better understand the mechanisms of the appearance and persistence of autoantibodies.

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