Research Square

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Investigation of Immune Microenvironment in Children with Langerhans Cell Histiocytosis

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Research article

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Abstract

Background: Langerhans cell histiocytosis (LCH) is a rare disease that mainly occur in children. The aim of this investigation is to explore the immune microenvironment of LCH and feasibility of immunotherapy for children with LCH.

Methods: Tissue samples were collected from 15 children with LCH and their clinical characteristics were recorded. The expressions of PD-1 ligand 1(PD-L1) and the presence of CD8 T lymphocytes were assessed by immunohistochemistry (IHC).

Results: Of the total 15 patients, 8 of them were PD-L1 positive and it accounted for 53.33% and 9 of them were CD8 T lymphocytes positive and it accounted for 60%. There were 8 out of the 15 patients that were both PD-L1 positive and CD8 positive and they accounted for 53.33%.

Conclusions: Our results showed that the expression of PD-L1 and presence of CD8 T lymphocytes occurred in the microenvironment of LCH. The findings indicated a possible new treatment option for LCH in children.

Background

LCH is a neoplasm in which CD1a+ / CD207 + dendritic cells have abnormal clonal hyperplasia (1, 2). LCH mainly occurs in children and the incidence is about 1 ~ 5 cases per million (3, 4). LCH is mainly caused by mutations in the *BRAF* and *MAP2K1* genes (5–7). These mutant genes belong to the RAS/RAF/MEK/ERK signaling pathway, suggesting that this pathway may involve in the pathogenesis of LCH.

The lesional microenvironment of LCH is characterized by infiltrate of immune cells such as T cells, macrophages and B cells (8, 9). The immune microenvironment therefore may produce an inflammatory response, causing organ damage. Studies have shown that the expression of immune cells (regulatory T cells, T helper cells and macrophages) in LCH is elevated, suggesting that the immune microenvironment plays an important role in LCH (10, 11).

Traditionally, chemotherapy and surgery are main treatment options for LCH in clinics. In recent years, significant progress has been made in immunotherapy. The immunotherapy has an advantage of long-lasting efficacy, high specificity, and low side effects when compared to current therapies (12). Immune checkpoint inhibitors such as Pembrolizumab and Nivolumab have been used in the treatment of malignant melanoma, non-small-cell lung cancer and other tumors (13–15). However, due to the complexity of tumor microenvironment, different patients have different clinical response to immune-therapy (16, 17). So far, studies on the immune microenvironment of LCH are very limited, especially in children. In order to explore the feasibility of immunotherapy for LCH in children, we analyzed the immune microenvironment by IHC to provide a better understanding of LCH.

Methods

Sample collection

This study is a retrospective study. All the recruited patients were histologically diagnosed as LCH. The tissue samples were taken from 15 children who underwent surgery from 2017 to 2020 at the Children's Hospital of Soochow University. This study was approved by the Ethics Committee of the Children's Hospital of Soochow University (No.2020CS086) and the need for consent was waived by the aforementioned ethics committee.

Immunohistochemistry

IHC of PD-L1 and CD8+ T lymphocytes

Tissue sections were dewaxed and repaired with Tris-EDTA (PH9.0), followed by PBS and peroxide block treatment. The recombinant anti-PD-L1 antibody (AB228415, abcam, Cambridge, MA, USA) was incubated overnight at 4 °C, the enzyme-labeled Polymer second antibody was incubated in an incubator for 30 min. After hematoxylin lining dying, color separation and sealing, the results were recorded.

Assessment of IHC

According to the protocol, a catalyzed signal amplification system kit (Boster, Wuhan, China) microarray was deparaffinized, rehydrated, and the antigens retrieved. Sections was then incubated with the antibody (Anti-CIP2A, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:100. The secondary antibody, developed using DAB, was conterstained with hematoxylin and observed under a microscope.

Double-blind IHC reading was investigated by 2 certified pathologists. Five visual fields were randomly selected under each microscope, and the percentage of positive cells was evaluated. The sample was considered positive for PD-L1 if 2+ intensity was observed in ≥5% of cells (18). The presence of any extent of tumor infiltrating CD8+ T lymphocytes was considered positive (19).

Results

Clinical characteristics

Clinical information of patients, including gender, weight and LCH subtype, was collected and summarized in Table 1. There were 9 males and 6 females. The ratio between the two was 3:2.

Table 1
Characteristics of study subjects

Patient	Gender	Weight(kg)	LCH subtype
P1	F	18	ss
P2	M	15	ss
P3	F	35	ss
P4	M	24	ss
P5	M	6.5	ms
P6	F	10	ss
P7	M	26	ss
P8	M	10.5	ss
P9	M	18	ss
P10	F	40	ss
P113	F	7.5	ss
P12	F	16	ss
P13	M	19	ss
P14	M	45	ss
P15	M	13	ss
SS: single system langerhans cell histocytosis; MS:multisystem Langerhans cell histocytosis			

Table 2
Summary of PD-L1 expression in langerhans cell histiocytosis

Score of PD-L1 expression	No. of cases	Percentage (%)
0	7	46.67
1	1	6.67
2	2	13.33
3	2	13.33
4	1	6.67
5	2	13.33

Table 3
Summary of CD8 T lymphocytes in langerhans cell histiocytosis

CD8 + TIL score	No. of cases	Percentage (%)
0	6	40.00
1	3	20.00
2	1	6.67
3	2	13.33
4	1	6.67
5	4	26.67

PD-L1 expression in LCH immune microenvironment

Seven patients (46.67%) had a PD-L1 expression with an IHC score of 0, 1 case (6.67%) with an score of 1, 2 cases (13.33%) with a score of 2, 2 cases (13.33%) with a score of 3, 1 case (6.67%) with a score of 4 and 2 cases (13.33%) of 5. The overall positive rate of PD-L1 was 46.66%.

Infiltration of CD8 + T cells in LCH immune microenvironment

Four patients (40.00%) had an IHC score of 0 on CD8 + T lymphocytes. There were 3 cases with a score of 1 (20.00%), 1 case with a score of 2 (6.67%), 2 cases with a score of 3 (13.33%), 1 case with a score of 4 (6.67%), and 4 cases (26.67%) with a score of 5. LCH patients were more common with mild infiltration of CD8 + T lymphocytes.

PD-L1 and CD8 expression in LCH immune microenvironment

Based on PD-L1 expression and the presence of mainly tumor infiltrating lymphocytes in tumor biopsies, 4 distinct tumor immune micro-environment subtypes were characterized (20): those with subtype T1 (PD-L1-, TIL-) and T4 (PD-L+, TIL-) are least beneficiary for anti-PD therapy; those with subtype T2 (PD-L1+, TIL+) account for most responses to anti-PD therapy; those with subtype T3 (PD-L1-, TIL+) can be converted into the more treatable subtype T2. The expressions of PD-L1 and CD8 in the immune microenvironment of each child with LCH were investigated. In this study, there were 8 cases of immune microenvironment with subtype 2 (53.33%), 3 cases of immune microenvironment with subtype 3 (20%), 4 cases of immune microenvironment with subtype 1 (26.67%), and 0 case of immune microenvironment with subtype 4 (0.00%). The details were listed in Table 4.

Table 4
Co-expression of PD-L1 and CD8 in langerhans cell histiocytosis

Patient	PD-L1	CD8+
P1	0	0
P2	0	0
P3	2	3
P4	1	1
P5	3	3
P6	0	1
P7	4	4
P8	0	0
P9	0	5
P10	2	1
P113	3	2
P12	5	5
P13	5	5
P14	0	5
P15	0	0

Discussion

LCH is a rare and proliferative neoplasm of histiocytic disease with clinical outcomes from good prognosis to mortality. We found CD8 + T infiltrating lymphocytes and PD-L1 expression in children's LCH immune microenvironment, suggesting that they may play a critical role in the development and progression of LCH.

The LCH contains inflammatory immune cells and several LCH studies have identified abnormal immune regulatory T cell subsets. High numbers of Foxp3 + regulatory T cells and gamma-delta T cells have been reported in patients with LCH, although their function remains unclear (21, 22). There were reports that cytotoxic T lymphocytes were observed in pulmonary LCH (23, 24). However, there have been no studies in children with LCH. Our study is the first one that focuses solely on the LCH immune microenvironment in children.

Treatment of LCH mainly includes surgery and chemotherapy. Chemotherapy has a huge adverse impact on children, both physically and psychologically. Targeted therapy is a newly emerged option for many patients with tumors. Vemurafenib targeting BRAF V600E has been proved effective in the preliminary study for LCH (25). However, the sustained action of targeted drugs is short and is prone to drug resistance (26). Therefore, there is an urgent need to explore a new treatment option that may provide an endurable effect and have less side effects for LCH patients, especially for children with LCH.

PD-1is an inhibitory T-cell costimulatory molecule. Tumor cells use PD-1/PD-L1 signaling pathway to inhibit cytotoxic activity of infiltrating T lymphocytes, creating an immunosuppressive microenvironment in which tumor cells escaped the immune attack. Recently, tumor immunotherapy has been widely used in treatment of some tumors. It has been reported that the 5-year survival rate of NSCLC treated with PD-1 antibody can be improved to 30–40% (27). But different patients respond differently, the response rate of NSCLC patients who were treated with Nivolumab antibody was 14.5%-19.4% (28). PD-L1 positive patients are significantly more effective than PD-L1 negative patients (29). In our study, there were subtype T2 (53.33%), subtype T1(26.67%), and subtype T3 (20.00%). Therefore the potential beneficiary from an immunotherapy is 73.33%, indicating immune-therapy may be a new treatment option and may be applied to children with LCH. Kemps et al reported the presence of substantial heterogeneity in CD8 + T cell density in LCH lesions, however, the authors doubt the efficacy of immune checkpoint inhibitor therapy in LCH through in silico analysis because predicted HLA class I binding and neopeptides derived from the BRAFV600E protein are not presented by HLA class I molecules (30). Further studies are warranted to verify the effectiveness of immunotherapy in children with LCH.

Conclusion

Our study for the first time explored the PD-L1 expression and CD8 + T lymphocytes simultaneously in children with LCH, providing a potential use of immune-therapy in the treatment for children with LCH.

List Of Abbreviations

IHC, immunohistochemistry

LCH, Langerhans cell histiocytosis

PD-1, Programmed cell death 1

PD-L1, PD-1 ligand 1

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Children's Hospital of Soochow University (No. 2020CS086) and the need for consent was waived by the ethics committee.

Consent for publication

All authors have approved the manuscript, approved the order of authors and agreed with its submission to *BMC Pediatrics*.

Availability of data and materials

All data and materials are available as requested.

Competing interests

The authors declare no conflicts of interest to disclose.

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Author contributions

FZ, WW, YL and XW conceived and designed the experiment; YZ, JD, LZ, PL, GS, JF and QY collected samples. YL and TZ conducted the immunochemistry for PD-L1 expression and CD8 T lymphocytes. All authors read, approved the manuscript and agreed to submit for publication.

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Figures

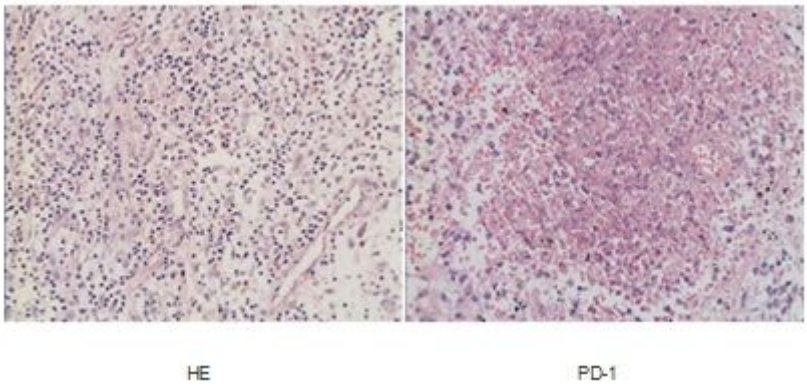


Figure 1

A representative image of PD-L1 expression in langerhans cell histiocytosis by Immunohistochemistry.

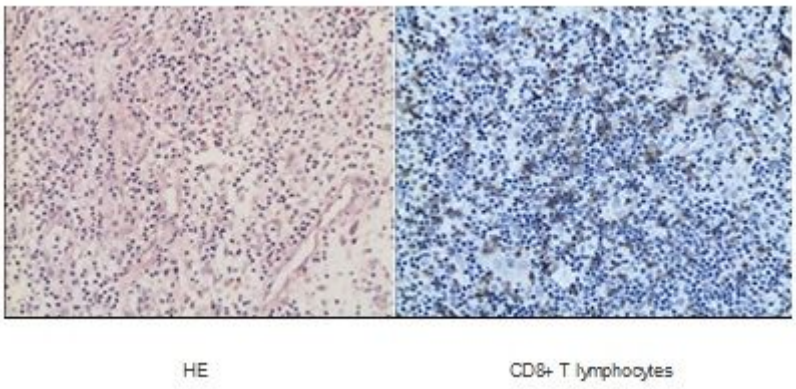


Figure 2

A representative image of CD8 T lymphocytes in langerhans cell histiocytosis by Immunohistochemistry.