Case Report

Characterization of an interdigitating dendritic cell hyperplasia case in a lymph node of a control C57BL/6 mouse

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Abstract: Interdigitating dendritic cell (IDC) hyperplasia is considered a benign spontaneous condition occasionally observed in the lymph nodes of mice. It has been rarely reported and, to the best of our knowledge, it has never been characterized using immunohistochemistry. The present work describes a spontaneous IDC hyperplasia case in a lymph node of a 16-week-old control female C57BL/6 mouse. Microscopically, the lymph node architecture was completely effaced by the proliferation of eosinophilic spindle cells with an abundant pale cytoplasm forming trabecule admixed lymphocyte infiltrates. The spindle cell population was positive for F4/80, partially positive for S100 calcium-binding protein A4 (S100A4), slightly positive for E-cadherin, and negative for α-Smooth muscle actin (SMA) and cytokeratin. Lymphocytes were positive for CD3, CD4, CD20 and negative for CD8. Spindle cells were considered to be originated from the myeloid lineage, based on the immunohistochemistry (IHC) results, but their precise origin remains unclear (IDC or macrophages); even if macrophage origin is most likely based on F4/80 positivity, this remains to be further clarified using other markers. (DOI: 10.1293/tox.2020-0039; J Toxicol Pathol 2021; 34: 101–106)

Key words: macrophages, interdigitating dendritic cells, F4/80, CD3, CD4, CD20

A 16-week-old, pathogen-free, C57BL/6 female control mouse from Janvier laboratory, presented with an enlarged and architecturally modified lymph node on histological examination. The animal was housed under controlled conditions (22 + 2°C of temperature, 45–65% relative humidity, 12 h:12 h light-dark cycle), fed a standard laboratory diet (SAFE A04C10) from Safe diet (Augy, France) and given access to water *ad libitum*. At scheduled necropsy, the mouse was anesthetized using isoflurane and then euthanized via exsanguination. Different tissues were harvested for histopathological evaluation, including the mesenteric lymph node (LN), spleen, and inguinal LN. At necropsy, no gross abnormality was identified, and the mouse was in an excellent body condition. Tissues were processed, histological sections were stained using hematoxylin and eosin (H&E) and analyzed by board-certified veterinary pathologists. The study and euthanasia were conducted according to the procedures of our laboratory and complied with the French and European Directives. Furthermore, the study was performed in accordance with the standards of the Ipsen Char-

ter on the Humane Care and Use of Laboratory Animals, including ethical review.

Histologically, the inguinal LN was increased in size (cross-section measuring approximately 5 mm), and its normal architecture was completely affected by a densely cellular and diffuse lesion compared to a normal LN (measuring 1 mm) (Fig. 1A and B). The cortex, paracortex, and medulla were replaced by homogenous sheets of lymphoid cells, separated by eosinophilic trabecule of eosinophilic spindle cells, giving the lymph node a marble appearance (Fig. 1C). Indeed, two major cell components were identifiable. The former consisted of round basophilic small lymphocytes, measuring 10 µm in diameter, with distinct cell borders with a scant cytoplasm, round, basophilic hyperchromatic nuclei, and indistinct nucleoli (Fig. 1D–F). Anisocytosis and anisokaryosis were minimal in this population. Intravascular lymphocyte "aggregates" were observed in the vascular lumens (Fig. 1F). The second population was composed of eosinophilic spindle cells measuring 40 µm in diameter with indistinct cell borders and an abundant eosinophilic cytoplasm (Fig. 1D and E). These cells had large pleomorphic nuclei, from elongated to round (depending on the sectioning), with finely stippled chromatin with few cells having enlarged nuclei and prominent nucleoli (up to 3). Anisocytosis was moderate in this population, and multinucleated cells were rarely observed (Fig. 1D, arrow). In both cell populations, no mitotic figure was observed. Focal proliferation extended into the peripheral sub-cutaneous adipose tissue.

Immunohistochemistry (IHC) analyses were per-

formed to characterize these two populations of cells. F4/80 is a mouse-specific macrophage marker expressed in most tissue macrophages[1–3]. It is expressed at low levels by blood monocytes and downregulated in macrophages in response to interferon (IFN)[4]. It is also expressed by Langerhans cells (LC) in the epidermis and is downregulated as they mature and leave the skin[5, 6]. In our control lymph nodes, F4/80 immunostaining was observed in macrophages in medullary cords and sinuses, in subcapsular sinuses and in rare cells in the mantle and T cell zones (IDCs were negative for F4/80).
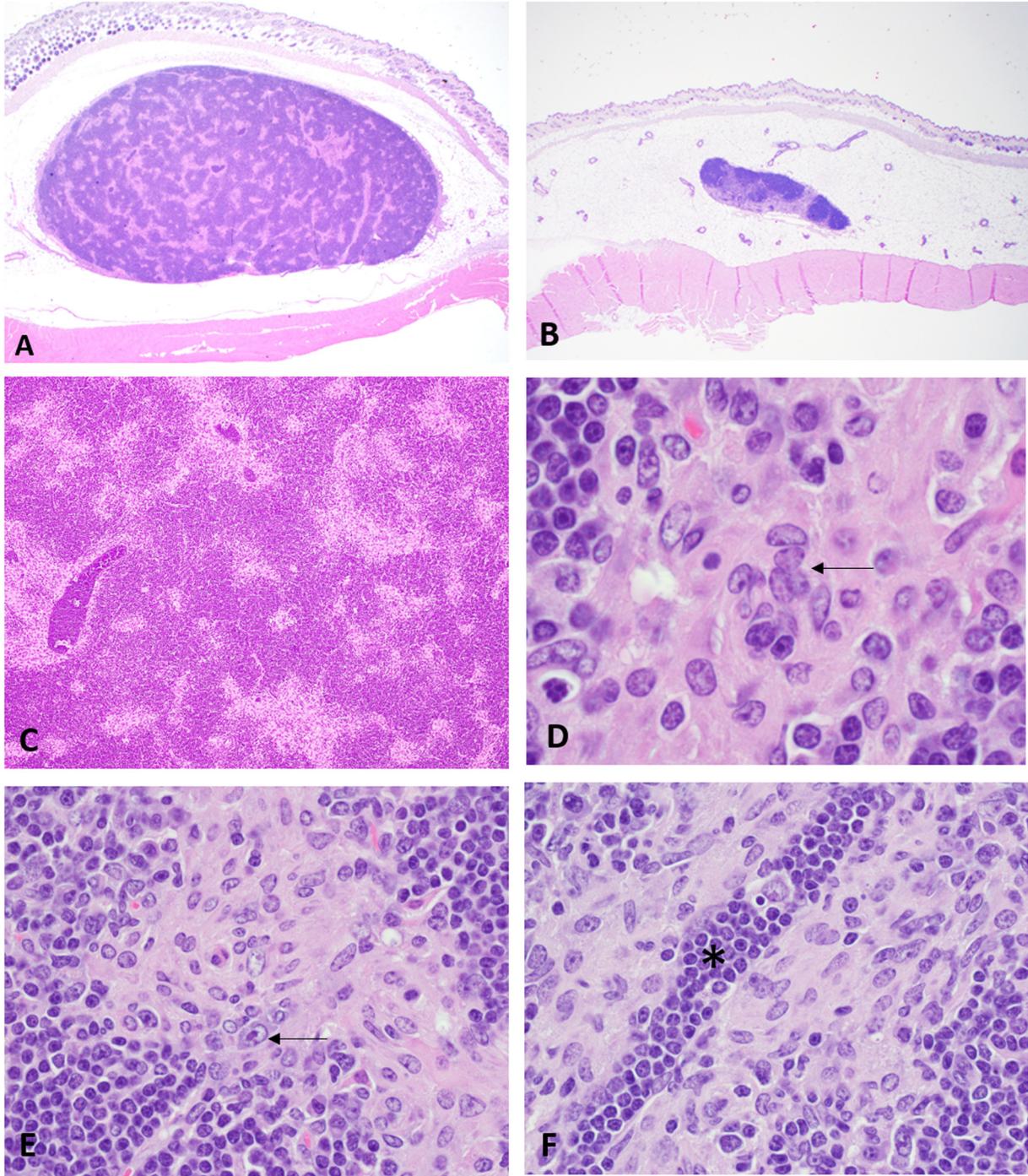
Fig. 1. A: Low magnification of the inguinal lymph node. The lymph node increased in size with the loss of a normal architecture. B: Normal inguinal lymph node. C: Eosinophilic trabecule of spindle cells admixed with regular sheets of small basophilic lymphocytes. D: Spindle cells with an abundant eosinophilic cytoplasm and rare multinucleate cells (arrow). E: Some spindle cells presented with nuclear pleomorphism with enlarged nuclei and prominent nucleoli (arrow). F: Intra-vascular lymphocyte aggregates (asterisk). Hematoxylin and eosin. Magnification: A, B: ×40; C: ×100; D: ×1,000; E, F: ×400.

In germinal centers, follicular dendritic cells (FDCs) and tingible body macrophages were negative for F4/80 (data not shown). There is partial discordance between our results and the literature regarding the expression of F4/80 positive macrophages in subcapsular sinuses (we observed few F4/80 macrophages in subcapsular sinuses, and they are supposed to be CD169+ F4/80−)[7]. In the literature, F4/80 expression is also described in other cell populations, such as immature dendritic cells (DCs), granulocytes, and some B cells[7]. S100A4 is expressed in monocytes and macrophages[8, 9], myeloid DCs[10], T cells[11], and other bone marrow-derived cells[12]. S100A4 promotes cell migration, epithelial-mesenchymal transition, motility, recruitment, and chemotaxis of macrophages[8]. Smooth muscle actin (SMA) and cytokeratin were used to rule out the smooth muscle or epithelial origin of spindle cells. Other antibodies were also used to characterize the lymphocyte populations (Table 1).

Normal LN and skin were used as positive controls for each marker, as well as isotypic controls for each primary antibody. Inhibition of peroxidase was performed with H_2O_2 at room temperature for 10 min. After treatment with 10% horse serum at room temperature for 30 min, the sections were incubated with primary antibodies at room temperature for 60 min. Then, secondary antibodies were used to detect the primary antibodies (horse anti-rabbit, anti-rat, or anti-goat diluted at 1/400 in Dako buffer, 30 min at room temperature). Signal amplification was carried out using the ABC system (30 min incubation at room temperature with streptavidin-horseradish peroxidase (HRP) Elite). Finally, positive reactions were visualized using 3,3-diaminobenzidine (DAB). Counterstaining was performed using aqueous hematoxylin and the slides mounted after dehydration.

The eosinophilic spindle cells reacted positively to F4/80, and some of them reacted positively to S100A4 (Fig. 2A and B). Some cells were slightly positive for E-cadherin and negative for smooth muscle actin and cytokeratin (data not shown). About 50% of lymphocytes were strongly positive for CD3, with most of them reacting positively to CD4, and 50% of the lymphocytes were positive for CD20 (Table 1, Fig. 2C–E). Intravascular lymphocyte aggregates presented with the same IHC phenotypes. Very rare lymphocytic cells were positive for perforin and CD8 (data not shown). In both populations, some spindle and lymphocytic cells were Ki-67-positive (proliferating cells) (Fig. 2F).

Based on the histopathologic observations, the lesion described here was consistent with a previously reported case of IDC hyperplasia. According to International Harmonization of Nomenclature and Diagnostic Criteria (IN-HAND) terminology "increased cellularity, all compartments" could have been used but, as the normal architecture of the tissue is completely effaced, this terminology does not seem adapted. IDC is the specific designation of dendritic cells located in the T-cell area of secondary lymphoid tissues (lymph nodes and spleen). This benign lesion is interpreted in the literature as a response to the presence of antigen in tissues drained by the lymph nodes[13]. Hyperplasia of IDC in paracortical T cell areas is generally seen in response to viral infection or irradiation. In the described case, the mouse was a control animal and did not show any clinical signs or macroscopic or microscopic evidence of chronic inflammation. Histologic evaluation of other tissues collected in this mouse did not show any lesions. A similar case is described on the website of the European Society of Toxicological Pathology (ESTP, https://www.eurotoxpath.

Table 1. Antibodies and IHC Characterization

Markers	References & Dilutions	Host and Clonality	Identified cell types	Spindle population	Lymphocyte population
CD3	Abcam ab16669, 1/100	Rabbit monoclonal	T cells	−	+
CD4	Thermo 149766 92, 1/100	Rat monoclonal	CD4+ T cells, some macrophages	−	+
CD8	Biorbyt orb10325, 1/100	Rabbit monoclonal	CD8+ T cells and CD8+ DCs	−	−
Perforin	Abcam ab16074, 1/300	Rat monoclonal	T CD8+ cells, NK cells	−	−
CD20	Abcam ab78237, 1/50	Rabbit monoclonal	B cells	−	+
F4/80 (Abcam)	Abcam ab6640, 1/300	Rat monoclonal	Mature macrophages, epidermal Langerhans cells, dermal DC, interdigitating cells (+/−)	+	−
S100A4 (Abcam)	Abcam ab197896, 1/1,000	Rabbit polyclonal	Monocytes, macrophages, T cells, DC	+/−	−
SMA (Abcam)	Abcam ab21027, 1/800	Goat polyclonal	Smooth muscle cells	−	−
E-Cadherin	Dako PA5-19479, 1/1,500	Rabbit polyclonal	Dermal DC	+/−	−
Pan-cytokeratin	Dako ZO622, 1/500	Rabbit polyclonal	Epithelial cells	−	−
Ki67	Abcam ab16667, 1/500	Rabbit monoclonal	Proliferating cells	+	+

org/guesswhat/index.php?id=case3). This was described as an incidental finding in a 20-week-old control male CD-1 mouse from a toxicity study. Contrary to the present case, the nodal lesion was focal and affected the mesenteric and mandibular lymph nodes. Furthermore, in this animal, a latent mouse hepatitis virus (MHV) infection was identified.
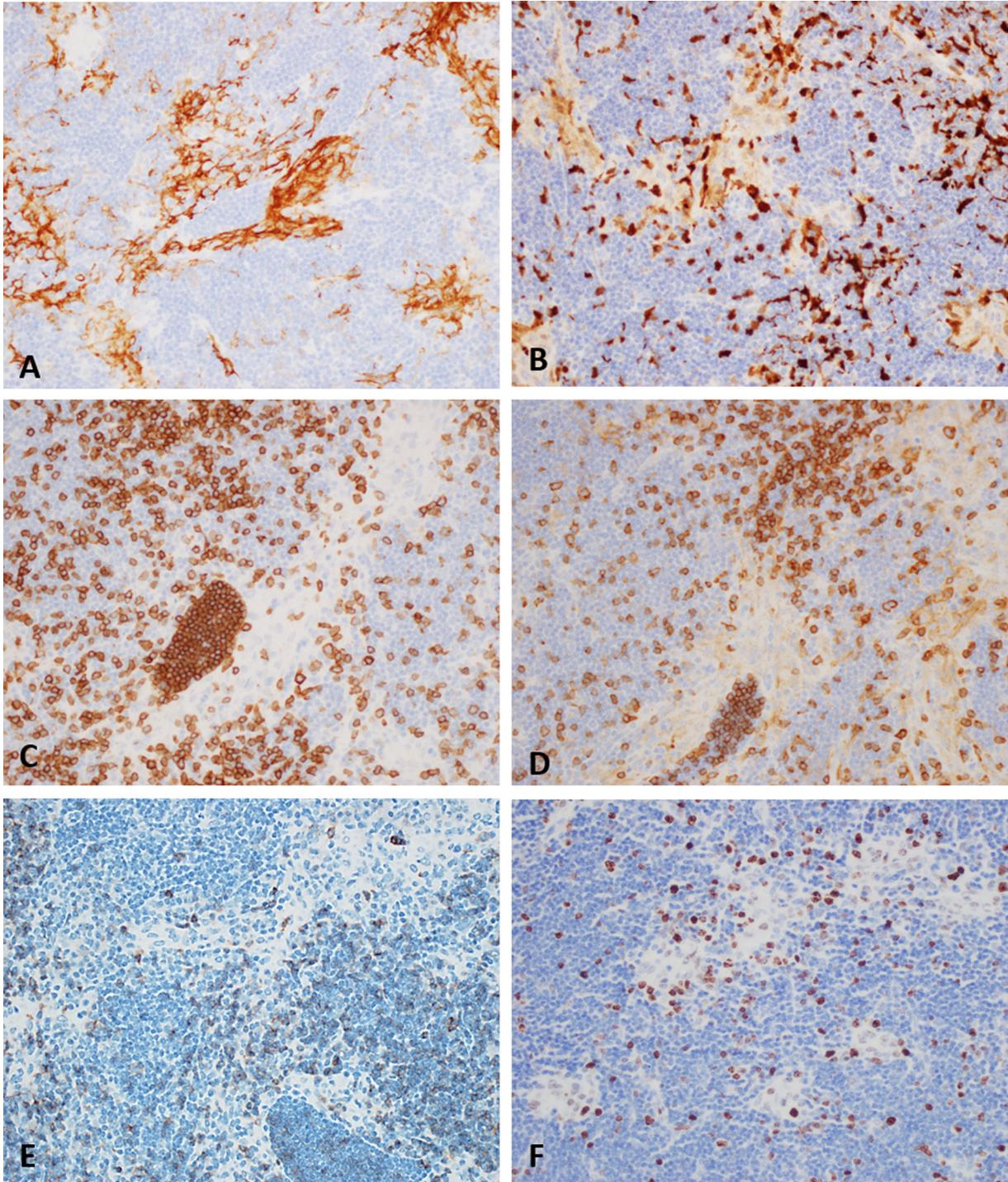
Fig. 2. Immunohistochemistry (IHC) analyses A: Cytoplasmic immunolabelling of F4/80 in the trabecule of spindle cells. F4/80 IHC, hematoxylin counterstain. B: Some F4/80 positive cells are also S100A4-positive. S100A4 IHC, hematoxylin counterstain. C: Membranous immunolabeling of CD3 in about 50% of the lymphoid population. CD3 IHC, hematoxylin counterstain. D: Membranous immunolabeling of CD4 in about 50% of the lymphoid population. Most of CD3-positive cells are also CD4-positive. CD4 IHC, hematoxylin counterstain. E: Membranous immunolabeling of CD20 in about 50% of the lymphoid population. CD20 IHC, hematoxylin counterstain. F: Nuclear immunolabelling for Ki67 in the histiocytic and lymphoid cellular population. Ki67 IHC, hematoxylin counterstain. Magnification: A–F: ×100.

The F4/80 positive spindle cells described in this case report are probably of the myeloid lineage, but other markers, such as CD11c, CD11b, class II major histocompatibility complex (MHC II), costimulatory molecules (CD80 or CD86), Fms-like tyrosine kinase 3 (Flt3), and Langerin, would enable a more precise identification of these cells. IDCs are of two origins: resident and migratory. Resident DCs enter the LN as precursors via the bloodstream and are positioned next to the LN filtering system to rapidly uptake soluble antigens[14]. Migratory DCs travel from tissue to LN via afferent lymphatics, carrying a high concentration of processed antigens to the LN paracortex and become IDCs[15]. In cutaneous lymph nodes, migrating DCs populate different nodal areas, depending on their subtype: Langerhans cells from the epidermis colonize the inner paracortex, whereas dermal DCs preferentially fill the outer paracortex[15]. In our case, migration of Langerhans cells is unlikely, given the absence of gross and microscopic skin lesions. However, a low expression of E-cadherin cannot rule out the possibility that these cells are migratory Langerhans cells (LCs), given that migration of LCs to lymph nodes is associated with a downregulation of this protein[16]. As the distinction between DC and macrophage populations is challenging, as markers frequently overlap[5, 17–19], the origin of the spindle cells in this case report remains difficult to identify. As a strong expression of F4/80 was identified, a macrophage origin of spindle cells is considered possible, but it remains to be demonstrated.

In the present case, only a subset of spindle cells was S100A4-positive, also pointing towards a migratory monocytic phenotype. About 50% of the lymphocytic population expresses T cell marker CD3, and 50% expresses B cell marker CD20. The heterogeneity of the lymphocytic population and the absence of lymphoma in other tissues did not support the hypothesis of an IDC hyperplasia-associated lymphoma[13]. The proliferation of follicular dendritic cells (FDC) from germinal centers can also be ruled out, as FDCs are negative for F4/80 and do not originate from the monocyte/macrophage lineage.

In mice, the differential diagnosis for interdigitating DC hyperplasia also includes histiocytic proliferative disorders, such as macrophage hyperplasia, sinus histiocytosis, histiocytic sarcoma (HS), and histiocyte-associated lymphoma (HAL)[13]. Sinus histiocytosis is characterized by the conservation of a normal lymph node architecture, with an accumulation of macrophages in the subcapsular and medullary sinuses, which was not observed in the present case[8]. HAL involves a large population of histiocytes with the accumulation of malignant lymphoid B or T cells[20]. In the present case, mixed B and T cell proliferation was observed, and the lymphoid cells did not present any evidence of malignant features. In LNs, HS is described as intra-sinusoidal histiocytosis in early lesions, progressing as unique or multiple infiltrating tumors. Early histiocytic sarcoma is morphologically similar to IDC hyperplasia, and these two can be confused.

In humans, a condition known as dermatopathic lymphadenopathy is characterized by an infiltration of dendritic cells in the paracortex. This condition is observed in patients with chronic dermatoses and mycosis fungoides and, to a lower extent, in patients with no appreciable skin disease. These dendritic cells are immunohistochemically characterized by being positive for S100, CD1a, and Langerin immunomarkers[21]. The restriction of dermatopathic lymphadenopathy to the paracortex area, the nodular distribution of hyperplastic cells, and the presence of melanophages are the main differences compared with dendritic cell hyperplasia observed in murine cases. Rosai-Dorfman-Destombes disease is a rare non-Langerhans cell histiocytosis described in children and young adults with multifocal severe lymphadenopathy and is characterized by the accumulation of activated S100+, CD68+, and CD1a− rounded histiocytes occurring in isolation or in association with autoimmune or malignant diseases[22]. This condition also differs from our murine case due to its histiocytic phenotype (large pale and round histiocytes), frequent emperipolesis, and multicentric, as well as possible extra-nodal, involvement.

In conclusion, IDC hyperplasia, considered as a rare benign condition of mouse lymph nodes, is characterized by the loss of a normal lymph node architecture and the proliferation of F4/80 positive eosinophilic spindle cells admixed with CD3+, CD4+, and CD20+ lymphocyte infiltrates. This lesion has to be distinguished from other more common lesions, such as macrophage hyperplasia, sinus histiocytosis, and histiocytic sarcomas. The reticular pattern and IHC staining are useful for distinguishing this particular lesion.

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