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Ontogeny of S-100 protein-positive histiocytes and lymphocytes in the human fetal lymphoreticular system.

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Abstract

In the human lymphoreticular system, the alpha and beta subunits of S-100 protein are found in ordinary monocyte-macrophages and non-phagocytic histiocytes such as Langerhans cells and interdigitating reticulum cells, respectively. The beta subunit is also present in some CD8+ T cells. In the present study, we investigated the ontogeny of these histiocytes and lymphocytes in humans. Yolk sacs and 4 to 21-week fetuses were examined immunohistochemically for the presence of S-100 protein subunits using antisera monospecific to each subunit. S-100 alpha + macrophages were present in the yolk sacs and the hepatic sinusoids of the 4th week embryos prior to bone marrow hematopoiesis. These macrophages later appeared in other lymphoid organs when anlagen of these organs were formed. No S-100 beta + cells were found in the yolk sacs. S-100 beta+ histiocytes were first detected in the hepatic sinusoids of the 5th week embryo, and after the 8th week of gestation, they were distributed in other lymphoid organs. S-100 beta+ lymphocytes were not found in the liver. They were first detected in the thymus at the 12th week of gestation, and were subsequently distributed in other lymphoid organs. These results suggest that S-100 beta+ lymphocytes and histiocytes may belong to different cell lineages, and the former may not be the precursor of the latter.

KEYWORDS: S-100 protein, ontogeny, lymphocyte, histiocyte

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In the human lymphoreticular system, the α and β subunits of S-100 protein are found in ordinary monocyte-macrophages and non-phagocytic histiocytes such as Langerhans cells and interdigitating reticulum cells, respectively. The β subunit is also present in some CD8$^+$ T cells. In the present study, we investigated the ontogeny of these histiocytes and lympocytes in humans. Yolk sacs and 4 to 21-week fetuses were examined immunohistochemically for the presence of S-100 protein subunits using antisera monospecific to each subunit. S-100 α^+ macrophages were present in the yolk sacs and the hepatic sinusoids of the 4th week embryos prior to bone marrow hematopoiesis. These macrophages later appeared in other lymphoid organs when anlagen of these organs were formed. No S-100 β^+ cells were found in the yolk sacs. S-100 β^+ histiocytes were first detected in the hepatic sinusoids of the 5 th week embryo, and after the 8 th week of gestation, they were distributed in other lymphoid organs. S-100 β^+ lymphocytes were not found in the liver. They were first detected in the thymus at the 12 th week of gestation, and were subsequently distributed in other lymphoid organs. These results suggest that S-100 β^+ lymphocytes and histiocytes may belong to different cell lineages, and the former may not be the precursor of the latter.

Key words : S-100 protein, ontogeny, lymphocyte, histiocyte

S-100 protein is an acidic calcium-binding protein with a molecular weight of approximately 20,000. It was first isolated from bovine brains by Moore (1). It was previously thought to be nervous tissue-specific (2, 3), but recent studies have shown that it is present in various nonneural cells including melanocytes and Langerhans cells (LC) of the skin (4), chondrocytes (5), adipocytes (6, 7), myoepithelial cells (6, 8), and interdigitating reticulum cells (IDC) of the lymphoid tissues (9).

It has been shown that S-100 protein is not a single protein, but a mixture of at least three similar proteins, S-100 ao, S-100 a, and S-100 b, with a respective sub-

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unit composition of $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$ (10, 11). Using monospecific antibodies to each subunit, we demonstrated the different distribution of these two subunits in human normal and neoplastic tissues (12). In the human lymphoreticular system, it was previously shown immunohistochemically that the α subunit of S-100 protein was present in blood monocytes and ordinary macrophages including Kupffer cells, but not in LC and IDC, while the opposite distribution was found for the β subunit (13). S-100 protein was also detected in peripheral blood T lymphocytes using an enzyme immunoassay (14). It was confirmed immunohistochemically that a small number of T lymphocytes possess the β subunit of S-100 protein (15). S-100 β subunit-positive T lymphocytes (S-100 β^+ T cells) have been shown to belong to the suppressor/cytotoxic subpopulation of T cells (16, 17). However, it has not yet been clarified when and where histiocytes positive to each S-100 subunit and S-100 β^+ T cells appear in human fetal lymphoreticular tissues. The purpose of this study was to investigate the ontogeny of such histiocytes and lymphocytes, particularly prior to bone marrow hematopoiesis, by an immunohistochemical method. Emphasis was placed on the question of whether S-100 α^+ and β^+ histiocytes are of different cell lineages and whether S-100 β^+ lymphocytes are precursors of S-100 β^+ histiocytes as stated by Watanabe *et al.* (18).

Materials and Methods

Antibodies. Affinity-purified rabbit antibodies monospecific for the S-100 α or β subunit were prepared as described previously (12, 13). A monoclonal antibody to leukocyte common antigens (LCA), which reacts mainly with lymphocytes in paraffin-embedded specimens, and rabbit antibodies against lysozyme, α_1 antitrypsin, and α_1 antichymo-

trypsin were purchased (DAKO, Denmark).

Tissues. Thymus, lymph node, spleen, liver, and bone marrow tissues from 31 human embryos and fetuses, ranging from the 4th to 21st week of gestation, and 15 human yolk sacs, ranging from the 4th to 7th week of gestation, were examined. Tissues from some newborns and adults were also examined as controls. The bone marrow was mainly obtained from vertebral bones. During the earlier gestational age, however, the femoral bone marrow was observed because hematopoiesis appeared first at the 16th week of gestation in the vertebral bone marrow and at the 12th week of gestation in the femoral bone marrow. The age of embryos and fetuses was determined from the time of the last menstruation. All tissues were fixed in 10% formalin, embedded in paraffin, and cut into 4.5 μm sections.

Immunohistochemical staining. After blocking endogenous peroxidase activity by treating with methanol containing 0.3% H_2O_2 for 30 min, dewaxed sections were stained by the peroxidase antiperoxidase (PAP) method (19) using rabbit antibodies against the S-100 α or β subunit (20 μg/ml), lysozyme (1 : 200), α_1 antitrypsin (1 : 100), or α_1 antichymotrypsin (1 : 100) as the primary antibodies or by the avidin-biotin complex (ABC) method (20) using a monoclonal antibody to LCA (1 : 20) as the primary antibody according to the staining procedure for Vectastain ABC kits (Ventor Laboratories, Burlingame, CA, USA). Double immunostaining was performed for the S-100 α subunit and lysozyme or for the S-100 β subunit and LCA. For double immunostaining, dewaxed sections were reacted with the first antibody and stained by the PAP or ABC (peroxidase conjugate) method. The peroxidase reaction was developed with 3, 3'-diaminobenzidine 4 HCl-H_2O_2 solution. After washing with phosphate buffered saline, the specimens were treated with the second antibody and stained by the ABC (alkaline phosphatase conjugate) method. The reaction product was developed with Fast blue BB salt.

Results and Discussion

S-100 α^+ macrophages. The result of S-100 α immunostaining is shown in Table 1. A small number of S-100 α^+ round cells

Table 1   Development of S-100 α^+ macrophages in fetal life[a]

Tissue	Gestational age (weeks)											After birth
	4	5	7	8	9	12	15	16	18	20	21	
Thymus		+	++	++	++	++	+++	+++	+++	+++	+++	+++
Spleen						++	+++	+++	+++	+++	+++	+++
Lymph node				+	+	++	+++	+++	+++	+++	+++	+++
Liver	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Bone marrow				++	++	++	++	+++	+++	+++	+++	+++
Yolk sac	+	+	++									

a: The number of positive cells is expressed as −, no positive cells; +, few positive cells; ++, moderate number of positive cells; +++, many positive cells. Blanks mean no development of the organ anlagen.

with abundant cytoplasm were detected in the capillaries of yolk sacs ranging from the 4th to 7th week of gestation. These cells were intermingled with other hematopoietic cells or attached to the capillary wall (Fig. 1). S-100 α^+ reticular or stellate cells were found also in the mesenchyme of yolk sacs. S-100 α^+ cells appeared in moderate number in the incomplete hepatic sinusoid (Fig. 2), but rarely were found in fetal vessels at the 4th week of gestation, at which time there were only a few mature erythroblasts and there was no apparent hematopoiesis in the liver. As gestation proceeded, S-100 α^+ cells were found in the thymus, lymph nodes, and spleen at the 5th, 8th, and 12th week of gestation, respectively, when their anlagen first appeared. These S-100 α^+ cells were thought to be macrophages for the following reasons: 1) Their tissue distribution and morphology were those of macrophages. 2) They often showed active phagocytosis. 3) Many lysosome-like granules were demonstrated in their cytoplasm by immunoelectron microscopy (data not shown). 4) Many of them were also positive for lysozyme, α_1 antitrypsin, and α_1 antichymotrypsin. 5) In the adult lymphoreticular system, the S-100 α subunit was detected only in cells of monocyte-macrophage lineage (13).

In the liver, mainly erythroblastic hematopoiesis began first in the extrasinusoidal space at the 5th week of gestation and some-

what later in the sinusoid. S-100 α^+ macrophages first appeared isolatedly in the sinusoid, but later appeared at the peripheral part of sinusoid as Kupffer cells. Macrophages present in the sinusoids intermixed with other hematopoietic cells were strongly positive for the α subunit, but Kupffer cells identified in the later stage of gestation and postnatal life were weakly positive. S-100 α^+ macrophages were detected in the bone marrow first at the 8th week of gestation, when the cavity of bone marrow was developed, but hematopoietic cells were not recognized. The number of S-100 α^+ macrophages in various organs increased rapidly, and their distribution after the 4th month of gestation was comparable to that in adult life (Fig. 3).

As to the origin of macrophages, the mononuclear phagocyte system theory proposed by van Furth et al. (21), which considers all macrophages to be the progeny of bone marrow-derived monocytes, is now generally accepted. However, recent studies on the histogenesis of fetal macrophages in humans and animals have revealed immature macrophages in the yolk sacs, hepatic sinusoids, and subepidermal mesenchymal tissues during early gestation prior to hematopoiesis in the bone marrow (22–27). These immature macrophages were called fetal macrophages and belonged to a cell lineage distinct from exudate macrophages derived from a progenitor cell in the
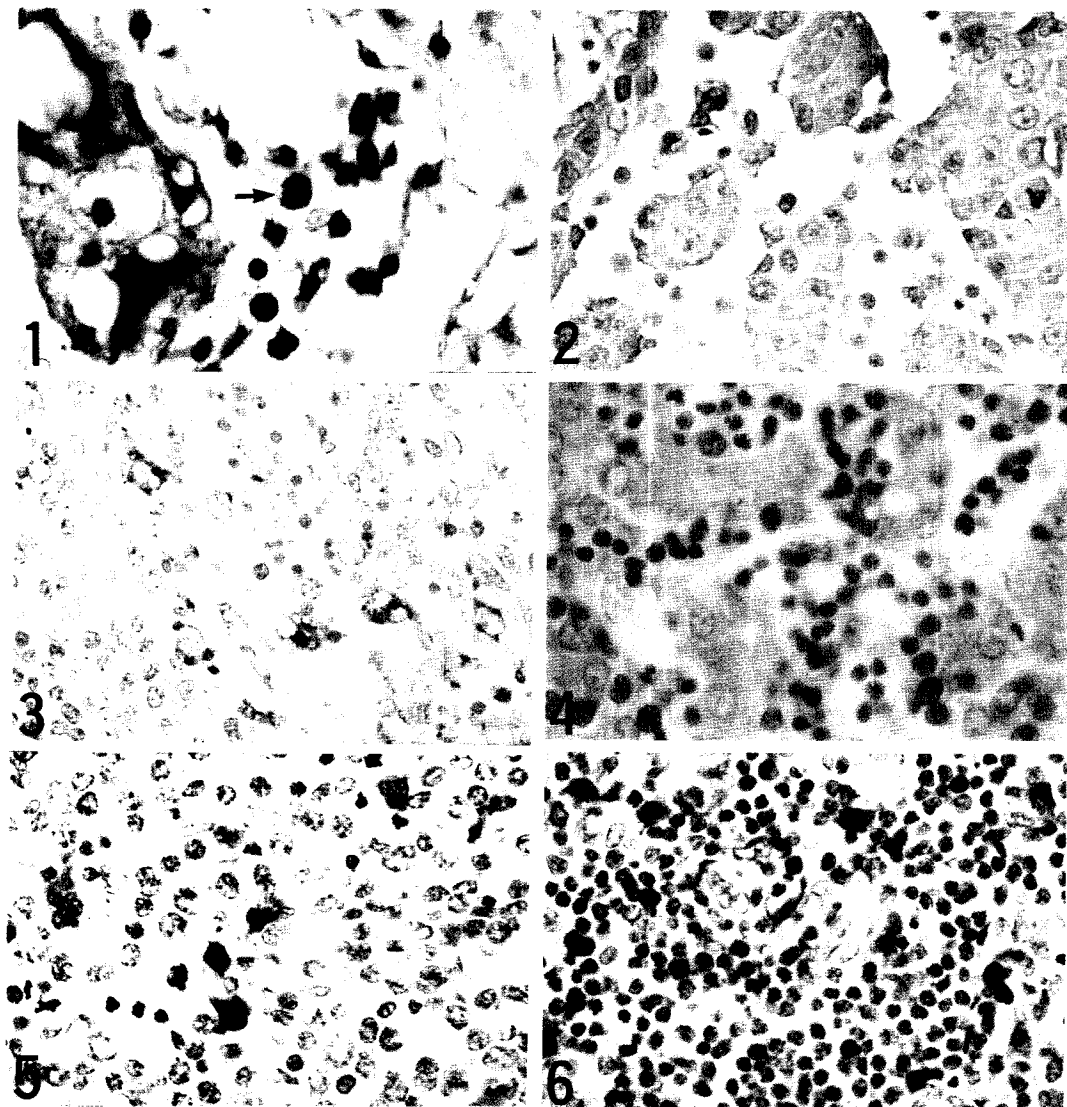
Fig. 1   An S-100 α^+ macrophage (arrow) in a blood capillary of the yolk sac at the 7th week of gestation. Endodermal cells were also positive for the α subunit.   ×960.

Fig. 2   S-100 α^+ macrophages in incomplete hepatic sinusoids of a 4-week embryo.   ×600.

Fig. 3   Many S-100 α^+ macrophages distributed diffusely in the spleen of a 21-week fetus. Note coarse granular immunoreaction products in the cytoplasm.   ×600.

Fig. 4   An S-100 β^+ cell in the hepatic sinusoid of a 5-week embryo. Note the elongated tortuous nucleus reacting positively.   ×600.

Fig. 5   Irregularly outlined S-100 β^+ histiocytes with long cytoplasmic processes in the thymic anlage of an 8-week embryo.   ×600.

Fig. 6   Many lymphoid cells and interdigitating reticulum cells positively stained for the β subunit in a lymph node of a 21-week fetus.   ×600.

bone marrow (27). In the present study, S-100 α^+ macrophages appeared first in the yolk sacs and hepatic sinusoids of the 4- week embryos, which were the youngest embryos examined and which hardly showed any hepatic hematopoiesis. The bone mar-

row cavities were first formed at the 8th week of gestation, and bone marrow hematopoiesis developed slightly in femurs only at the 12th week of gestation. These results strongly suggest that S-100 α^+ macrophages originate in the blood islands of yolk sacs at the early stage of gestation, and via fetal circulation move to the hepatic sinusoids and mesenchymal tissues of the whole body. Besides S-100 α^+ macrophages, immigrant cells may include hematopoietic stem cells which settle in the hepatic sinusoids and become the second source of macrophages in the later gestational stage. Therefore, the yolk sac and fetal liver may be the major source of tissue macrophages before bone marrow hematopoiesis develops.

S-100 β^+ histiocytes. The result of S-100 β immunostaining is shown in Table 2. S-100 β^+ cells were first detected in the hepatic sinusoid at the 5th week of gestation and were continuously present until the 21st week of gestation, but only in a small number (Fig. 4). Although fetal livers after the 21st week of gestation were not examined, no S-100 β^+ cells were detected in the hepatic sinusoids of newborns and adults. S-100 β^+ cells had elongated tortuous nuclei and cytoplasmic processes and were nega-

tive for lysozyme, α_1-antitrypsin, and α_1-antichymotrypsin. They were thought to belong to the same cell lineage as IDC of adults. At the 8th week of gestation, these S-100 β^+ histiocytes were detected in the thymic (Fig. 5) and lymph node anlagen. In the former, they tended to appear mainly in the medulla and the border of the cortex and medulla at later stages of gestation when the cortex and medulla became distinct. In the spleen, they were first detected at the 12th week of gestation when the splenic anlage arose. As gestation proceeded, S-100 β^+ histiocytes increased in number and outnumbered those in newborns. In the bone marrow, a considerable number of S-100 β^+ cells were recognized after the 16th week of gestation, but it was very difficult to identify the species of positive cells because some polymorphonuclear leukocytes were positively stained.

S-100 β^+ histiocytes such as LC and IDC have been thought to be histogenetically derived from the progenitors in the bone marrow and to belong to the mononuclear phagocytic system (28, 29). The present study, however, indicated that S-100 β^+ histiocytes exist in the hepatic sinusoid,

Table 2 Development of S-100 β^+ histiocytes and lymphocytes in fetal life[a]

Tissue	Cell	Gestational age (weeks)											After birth
		4	5	7	8	9	12	15	16	18	20	21	
Thymus	Histiocytes	−	−	++	+++	+++	+++	+++	+++	+++	+++	+++	++
	Lymphocytes	−	−	−	−	+	++	++	++	++	+++	++	+
Spleen	Histiocytes						+	++	++	++	++	+++	++
	Lymphocytes						−	−	−	+	+++	++	+
Lymph node	Histiocytes				++	++	++	+++	+++	+++	+++	+++	++
	Lymphocytes				−	−	+	++	++	++	+++	+++	+
Liver	Histiocytes	−	+	+	+	+	+	+	+	+	+	+	−
	Lymphocytes	−	−	−	−	−	−	−	−	−	−	+[b]	−
Yolk sac	Histiocytes	−	−	−									
	Lymphocytes	−	−	−									

a: The number of positive cells is expressed as −, no positive cells; +, few positive cells; ++, moderate number of positive cells; +++, many positive cells. Blanks mean no development of the organ anlagen.

b: This fetus suffered from cytomegalic inclusion disease, and a small number of S-100 β^+ lymphocytes were found in lymphoid cell aggregates associated with cytomegalic inclusion bodies.

thymus, and lymph node prior to bone marrow hematopoiesis, and do not arise from blood monocytes as do S-100 α^+ fetal macrophages. S-100 β^+ cells were not detected in the yolk sac, but it does not necessarily deny the possibility of the presence of S-100 β^- precursors in the yolk sac. The tissue distribution and the time of appearance of S-100 β^+ histiocytes were quite different from those of S-100 α^+ macrophages, suggesting that these two kinds of histiocytes (macrophages) apparently belong to different cell lineages. Nevertheless, they might have a common stem cell or progenitor because monocyte colony forming cells of human bone marrow have the phenotypes of both phagocytic macrophages and LC/IDC (30).

S-100 β^+ lymphocytes. Small S-100 β^+ lymphoid cells with a round chromatin-rich nucleus and scanty cytoplasm were first detected in the thymic cortex and lymph node of the 12-week embryos, intermingled with S-100 β^+ histiocytes. As gestation proceeded, S-100 β^+ lymphoid cells increased in number (Fig. 6) and were distributed in the lymphoreticular tissue of the whole body, appearing in the spleen at the 18th week of gestation. They were positive for LCA in contrast with S-100 β^+ histiocytes which were negative for LCA by double immunostaining (Fig. 7). In the yolk sacs, there were no S-100 β^+ cells at any stage of gestation. S-100 β^+ lymphocytes were much more frequently detected in the fetuses than in the postnatal specimens.

In adults, S-100 β^+ lymphocytes, which possess the CD8 phenotype (16, 17), are present in the peripheral blood (15) and T-zone area of lymphoid organs in close association with S-100 β^+ histiocytes (31). Also in fetuses, they were found together with S-100 β^+ histiocytes. Watanabe *et al.* (18) suggested that small S-100$^+$ lymphoid cells with round or cerebriform nuclei coexisting with S-100$^+$ histiocytes might be precursors of the latter. However, this may not be the
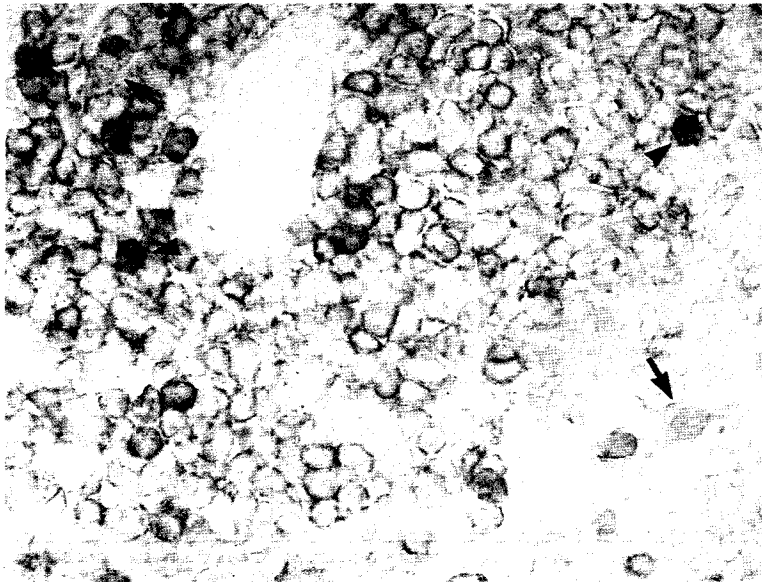
Fig. 7   Double immunostatning for S-100 β subunit (brown) and leucocyte common antigens (blue). Note doubly stained lymphoid cells   (arrow heads)   and interdigitating reticulum cells positive only for the S-100 β subunit (arrows). Spleen of a   20-week   embryo.   ×600.

case because S-100 β^+ histiocytes appeared far earlier than S-100 β^+ lymphocytes in various tissues, and they had different phenotypic characters. Furthermore, only S-100 β^+ histiocytes were found in the hepatic sinusoids. Therefore, S-100 β^+ histiocytes and lymphocytes may belong to different cell lineages although the latter take the dendritic form in the presence of 12-0-tetradecanoylphorbol-13-acetate (TPA)(Takahashi *et al.* in preparation). No S-100 β^+ lymphoid cells were found in the yolk sac or fetal liver sinusoid, suggesting that the stem cell or precursor of S-100 β^+ lymphocytes may be negative for the β subunit of S-100 protein.

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