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Definitive Hematopoiesis from Endothelial Cells in the Mouse Embryo; A Simple Guide

Daisuke Sugiyama[1] and Kohichiro Tsuji*

Circulation is composed of two interactive systems, the cardiovascular and the hematopoietic, which affect each other. Recently, endothelial progenitor cells/angioblasts have been identified in the circulation of the adult mouse and human. Furthermore, some hematopoietic cells (HCs) have been shown to contribute to angiogenesis, suggesting that HCs can transdifferentiate into endothelial cells (ECs). Although these concepts in adult are still controversial, understanding the mechanisms of the relationship between ECs and HCs would benefit the clinical application for cardiovascular and hematologic disorders. Both ECs and HCs are considered to be derived from a common germ layer, the mesoderm, and have more intimate relationship in embryo than in adult. Here, we describe the relationship between ECs and HCs with special attention to the hemogenic ECs in the mouse embryo. (Trends Cardiovasc Med 2006;16:45–49) © *2006, Elsevier Inc.*

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• General Hematopoietic Cell Development

The relatively short gestation period of the mouse (18–21 days) contributes to its suitability as a research model in the field of mammalian development. In mice, hematopoiesis begins in the extraembryonic yolk sac (YS) at 7.5 days postcoitum (dpc), shifting to fetal liver (FL) at midgestation, then to spleen, and finally to bone marrow (BM) shortly before birth (Figure 1) (Dzierzak et al. 1998).

Earlier studies suggested that hematopoietic cell (HC) development initiated at one time in the YS. However, nonmammalian embryo grafting experiments suggested that there is another origin of hematopoiesis, which was shown to be the intraembryonic paraaortic splanchnopleura (P-Sp)/aorta-gonad-mesonephros (AGM) region at 8.0 to 11.5 dpc. The P-Sp/AGM region is now considered by some to be the major source of adult hematopoiesis, and YS is considered a possible secondary source, although this concept is still controversial. Recently, the placenta has been identified as yet one more hematopoietic organ and a niche for the immature HC pool at 12.5 dpc. The hematopoietic activity of the placenta is transient and diminishes after the FL hematopoiesis becomes active. It is of interest whether the placenta produces HCs de novo and how the placenta affects the maintenance of HCs. Further studies will be necessary in the future. (Current work in this area has been reviewed by Mikkola et al. (2005)).

• Primitive and Definitive Hematopoiesis

One problem in the field of hematopoiesis research is that the terminology is historically complicated. There are two types of hematopoiesis, primitive (embryonic) and definitive (adult). Primitive hematopoiesis is transient, whereas definitive hematopoiesis continues until death. Avian embryos were first used to identify the origin of hematopoiesis, and several observations were applied to mouse embryos, although avian hematopoiesis and murine hematopoiesis are not identical. Primitive hematopoiesis is mainly restricted to erythropoiesis taking place in the YS. Some well-known characters of the cells have been used specifically for definitive hematopoiesis: (a) definitive erythroid cells, (b) high-proliferative potential colony-forming cells, (c) lymphoid cells, (d) hematopoietic stem cells (HSCs), and (e) others (granulocyte-macrophage colony-forming cells, spleen colony-forming cells, etc) (Figure 2). Although all characters are for identification of definitive hemato-

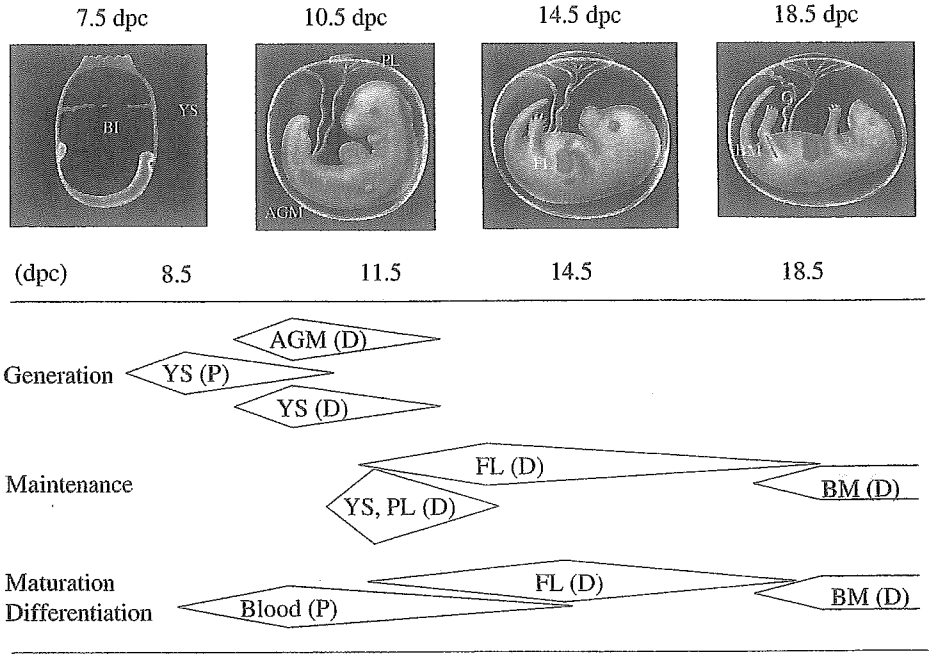

Figure 1. Schema of embryonic HC development. BI, blood island; PL, placenta; P, primitive hematopoiesis; D, definitive hematopoiesis.

poiesis, they are of unequal hierarchy. Thus, whereas HSCs have the potential to proceed to any mature blood cell during definitive hematopoiesis, other cells, such as definitive erythroid cells, are of more limited potential. In this context, definitive hematopoiesis is composed of two waves of emergence. The first wave is mainly definitive erythropoiesis, which takes place in the YS and diminishes gradually until birth, as if to cover the gap between primitive erythropoiesis in YS and definitive erythropoiesis by HSCs colonized in FL to support the growing embryo. Hematopoietic stem cells, generated in the P-Sp/AGM region and probably to lesser extent in the YS, are supposed to colonize the FL, initiate the second wave of definitive hematopoiesis, and sustain BM hematopoiesis in adult life. New terminology that distinguishes between these two waves of definitive hematopoiesis will be necessary to avoid confusion.

- **The Relationship Between ECs and HCs: Hemangioblasts and Hemogenic ECs**

Based on morphologic observation, the existence of "hemangioblasts" and "hemogenic ECs" has been hypothesized. Hemangioblasts are the cells capable of differentiating into both ECs and HCs in both primitive and definitive hematopoiesis, whereas hemogenic ECs are structurally ECs and have only definitive hematopoietic potential. The first hematopoietic site in the YS is called a blood island, in which HCs (mainly primitive erythroid cells) develop inside and in close association with the outer layer of ECs. Current work in this area has been reviewed by Ferkowicz and Yoder (2005). As the embryos develop, the EC layer forms blood vessels and HCs are released into the circulation. The spatial and temporal proximity of both EC and HC development suggested the possibility that both lineages were derived from a common precursor, the hemangioblast. Evidence supporting existence of such a cell comes from gene targeting experiments that result in the absence of both lineages. In addition to the fact, several markers (e.g., Flk-1, CD31, CD34, Tie-2) are expressed in both lineages and embryonic stem cell technology has yielded insights for hemangioblast. On the other hand, a different model of EC and HC development states that HCs develop from ECs and, in terms of hematopoietic clusters of cells that are attached to the ventral lumenal aspect of the dorsal aorta, are observed at 10.5 dpc (Garcia-Porrero et al. 1995, Marshall and Thrasher 2001). Therefore, it had long been proposed that hematopoiesis might originate from a specific subset of vascular ECs named hemogenic ECs. These clusters are observed from fish to human, regardless of the species. Especially in mammals, they are also observed in the omphalomesenteric artery and umbilical artery as well, both of which have HSC potentials (Garcia-Porrero et al. 1995, De Bruijn et al. 2000). The existence of hemogenic ECs has already been demonstrated in the YS as well as in the P-Sp/AGM region, and several lines of evidence support the notion that hemogenic ECs are able to generate only definitive HCs (Nishikawa et al. 1998, Sugiyama et al. 2003, Yokomizo et al. 2001). Although these two terms have been used interchangeably, they have distinct meanings. It is still unclear whether so-called hemangioblasts have potentials capable of differentiating into the other mesodermal lineages (i.e., skeletal muscle, bone, cartilage, and adipose tissue). Recently, a brachyury+Flk-1+ cell, which is supposed to be a hemangioblast, was detected in the posterior primitive streak in the mouse embryo at 7.5 dpc (Huber et al. 2004).

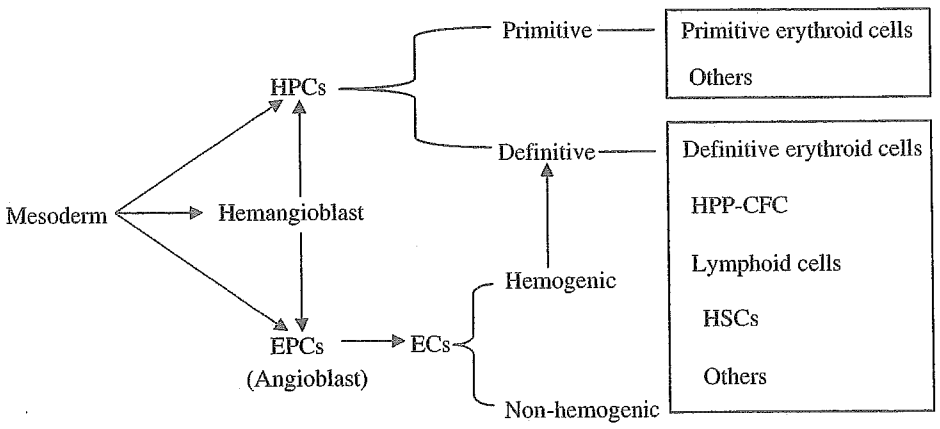

Figure 2. Simplified schema of hematopoietic differentiation pathway and type of hematopoiesis.

◦ Definitive Hematopoiesis in YS

The YS at 8.25 dpc has a potential of definitive erythropoiesis, in which the adult type hemoglobin gene is identified by RT-PCR (Palis et al. 1999). VE-cadherin+CD45− cells, which are supposed to be ECs, were isolated from the YS and exhibited lymphoid potential in vitro and in vivo (Nishikawa et al. 1998, Fraser et al. 2002). The cells in the YS at 9.5 dpc have the potential of HSCs only when transplanted into neonate recipients conditioned by busulfan (Yoder et al. 1997). The cells in the YS at 8.0 dpc, before the establishment of circulation, have a potential of HSCs only when cocultured with the AGM-derived stromal cell line (Matsuoka et al. 2001). Taken together, this has proved that the YS has a potential of both waves of definitive hematopoiesis as mentioned in the previous section. According to the avian in vivo study, the YS contributes to definitive erythropoiesis, but not to lymphopoiesis and HSCs (Dieterlen-Lievre and Le Douarin 1993), suggesting that we might overestimate the hematopoietic potential by an in vitro assay. Difficult accessibility of mammalian embryos has made investigators establish some assay methods to identify the hematopoietic potential, which does not reflect the hematopoietic fate. Some in vitro assay method might enable the cells to acquire the unexpected potential that does not reflect the cell fate. To address this issue, we have designed experiments with the use of a whole-embryo culture system that enables us to manipulate the mouse embryo and follow the cell fate in vivo. Although the YS has a potential of definitive hematopoiesis, it remained unclear that hemogenic ECs in the YS contribute to definitive hematopoiesis in vivo in mice. DiI-conjugated acetylated low-density lipoprotein (Ac-LDL-DiI), which is incorporated into ECs and macrophages, was inoculated into embryos at 10.0 dpc by intracardiac injection, followed by whole-embryo culture (Figure 3) (Sugiyama et al. 2003). One hour after Ac-LDL-DiI inoculation, DiI staining was found along the entire endothelial tree. In sections, DiI staining was observed in the endothelial layer. The DiI+ cells expressed CD31 and CD34 but not CD45 by flow cytometry, which is an antigen characteristic for the endothelial lineage. Twelve hours after culture,

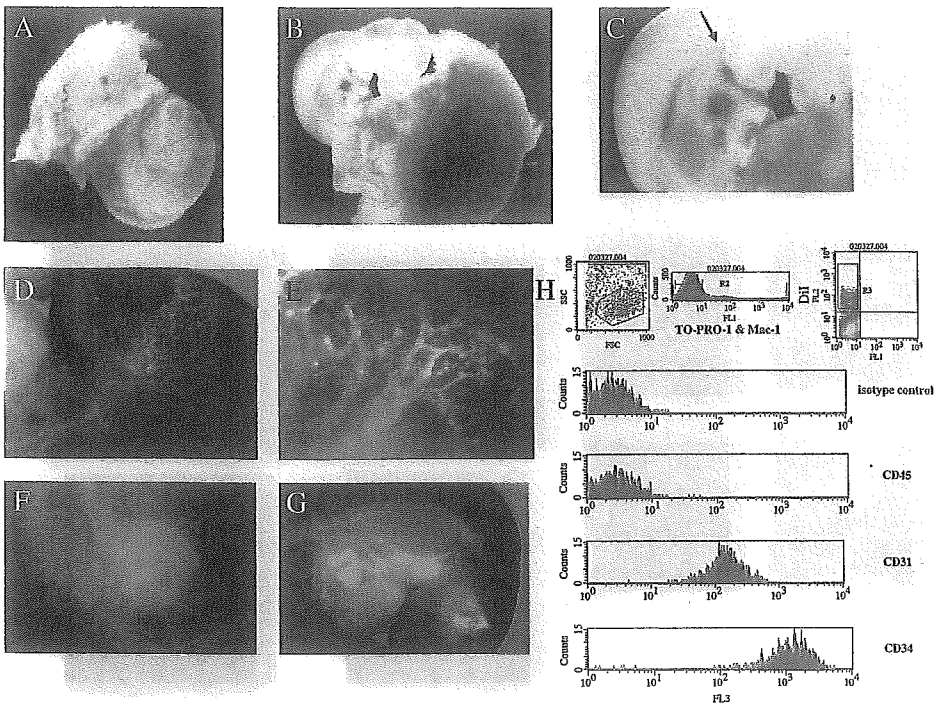

Figure 3. Ac-LDL-DiI inoculation by intracardiac injection. (**A**) Embryo at 10.0 dpc is dissected out without damage to the embryos. (**B**) YS is cut along blood vessels and embryo is bared. (**C**) Ac-LDL-DiI was inoculated by intracardiac injection. Arrow shows the glass needle. (**D**-**G**) DiI illumination (yellow) detected along blood vessels of whole body 1 hour after Ac-LDL-DiI inoculation (**D**, head part; **E**, magnified view of **D**; **F**, heart part; **G**, truncal part). (**H**) FACS profile of the cells incorporating Ac-LDL-DiI 1 hour after inoculation. After eliminating the dead cells and macrophages, DiI+ cells were examined. DiI+ cells have an endothelial character (CD45−CD31+CD34+).

definitive erythropoiesis considered to be from hemogenic ECs incorporating Ac-LDL-DiI was identified, although we could not tell which ECs contributed this hematopoiesis, as Ac-LDL-DiI was inoculated into the whole body of the embryo. Taken together with the report that the receptor of erythropoietin, a pivotal cytokine for definitive erythropoiesis, is expressed in the YS vasculature, suggesting that this definitive erythropoiesis is generated through ECs in the YS (Lee et al. 2001), the definitive erythropoiesis of hemogenic EC origin seems to be conserved between mouse and chicken. Recently, it has been shown that Tie-2 + Flk-1dimCD41− cells, which are supposed to be ECs in the YS at 8.25 dpc, give rise to HCs expressing CD41, an HC marker, and acquire the potential of definitive hematopoiesis only when cocultured with OP9 stromal cell lines, suggesting the existence of hemogenic ECs (Li et al. 2005). Although some groups claim that the YS has no potential of lymphopoiesis and HSCs, negative results might be due to the problem in the assay systems. The potential of definitive lymphopoiesis and HSCs may remain inactivated before 10.5 dpc and specific condition might be required for the YS to acquire the potentials of definitive lymphopoiesis and HSCs.

◦ Definitive Hematopoiesis in P-Sp/AGM Region

A series of studies has demonstrated that the P-Sp/AGM region has potentials of lymphopoiesis and HSCs (Cumano et al. 1996, 2001, Matsuoka et al. 2001, Medvinsky and Dzierzak 1996). VE-cadherin+CD45− ECs isolated from the P-Sp/AGM region exhibit lymphoid potential like those from the YS (Nishikawa et al. 1998). Endothelial cells expressing *Runx-1*, an essential transcription factor for definitive hematopoiesis in the P-Sp/AGM region, have a potential of adult repopulating HSCs (North et al. 2002). The first expression of *Ly-6A*, one of the earliest genes expressed in HSCs, is restricted to the cells inserted into the endothelial layer in the P-Sp/AGM region (de Bruijn et al. 2002). Although we have shown that

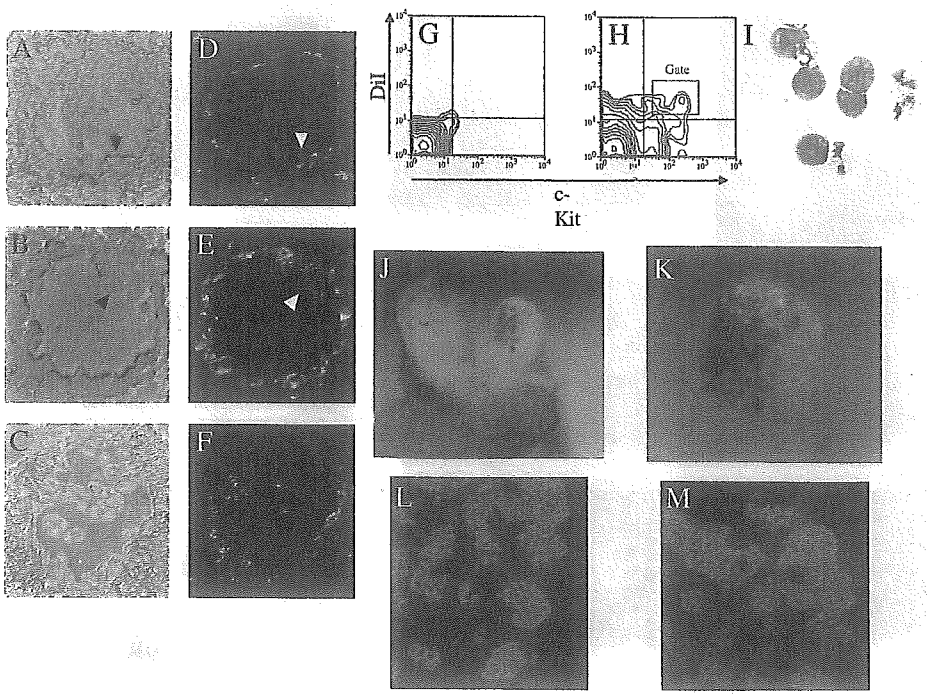

Figure 4. Characterization of DiI+ cells 12 hours after Ac-LDL-DiI inoculation. (**A-E**) Twelve hours after inoculation, DiI+ hematopoietic clusters were observed in the dorsal aortic floor (**A**, phase contrast; **D**, fluorescence) and in the umbilical artery (**B**, phase contrast; **E**, fluorescence). Arrowheads show the hematopoietic clusters. DiI+ circulating HCs were observed in the omphalomesenteric artery (**C**, phase contrast; **F**, fluorescence). (**G,H**) The blood sample of inoculated embryos was examined by flow cytometry. (**G**) Isotype control. (**H**) x-Axis, c-Kit; y-axis, DiI. Sorting gate for c-Kit+DiI+ cells is shown. (**I**) The morphology of c-Kit+DiI+ cells sorted with the use of the gate shown in (**H**). (**J,K**) The DiI+ cells sorted out from the blood of inoculated embryos were introduced back into the circulation of the second recipient embryos. (**J**) Fetal liver lobes of the recipient embryo. DiI+ cells that colonied FL were observed as yellow color (**K**). Magnified view of (**J**). (**L,M**) Observation by confocal microscopy. The colonized DiI+ cells were observed as white color.

Ac-LDL incorporating ECs contribute to definitive erythropoiesis, the HSC potential remained unclear. To address this issue, we further characterized the HCs generated from Ac-LDL incorporating ECs. Twelve hours after inoculation, DiI staining was observed in the hematopoietic clusters protruding into the aortic lumen and only a few circulating cells in addition to endothelial layer in the P-Sp/AGM region (Figure 4) (Sugiyama et al. 2005). Similar findings have already been observed in chicken embryos (Jaffredo et al. 1998). We sorted out the DiI+c-Kit+ cells from circulation by flow cytometry, which revealed two distinct populations by morphologic observation: immature HCs/HPCs with a blastic cell aspect and macrophages (Figure 4). In addition, the sorted DiI+ cells showed potentials of both neonate and adult HSCs by transplantation experiments and could colonize the FL when introduced back into recipient embryos (Figure 4) (Sugiyama et al. 2005). Because Ac-LDL-DiI is inoculated into whole body of embryos, it could not be identified which hemogenic ECs contributed to the HSCs. Taken together with the previous reports, hemogenic ECs incorporating Ac-LDL-DiI in the P-Sp/AGM region and probably in the YS contribute to neonate and adult HSCs.

• EC-Derived Definitive Hematopoiesis

Hemogenic ECs could be detected in both the YS and P-Sp/AGM region after 9.5 dpc (Nishikawa et al. 1998). Recently, hematopoietic clusters have been shown in the YS at 9.5 dpc as well as in the P-Sp/AGM region (Li et al. 2005, Yokomizo et al. 2001). The timing of hemogenic EC emergence is consistent with the emergence of hematopoietic clusters in both the YS and P-Sp/AGM region, suggesting that the hematopoietic clusters originate from hemogenic ECs (Garcia-Porrero et al. 1995, Marshall and Thrasher 2001). Although we showed that both waves of definitive hematopoiesis are generated by Ac-LDL incorporating ECs, we could not deny the possibility that the other cells might somehow contribute to definitive hematopoiesis. Recently, subaortic patches (SAPs) consisting of mesenchymal cells underlying the dorsal aortic floor have been shown to have a potential of definitive hematopoiesis in the P-Sp/AGM region at 10.5 dpc (Bertrand et al. 2005). Cells found in both the SAPs and hematopoietic clusters express AA4.1, CD31, CD41, *GATA-3*, *GATA-2*, and *Runx-1*, but not CD45. Because mesenchymal cells within SAPs express many of the same markers as HSCs, and also have definitive hematopoietic potential, the authors suggested that hematopoietic clusters are formed by the cells from SAPs migrating through the ECs of the dorsal aorta rather than formed by hemogenic ECs themselves (Bertrand et al. 2005, North et al. 2002). However, electron microscopic observation shows that hematopoietic clusters attach strongly to the ECs by tight junction. It is unlikely that the hematopoietic clusters in the umbilical artery originate from a non-EC component structurally.

It is important to remain aware that, as our understanding of hematopoietic and vascular development improves, new facts will require us to reevaluate existing definitions so that we may adapt them appropriately. For example, CD45 is a panleukocyte marker supposed to be expressed in hematopoietic clusters. However, the intensity of CD45 expression in

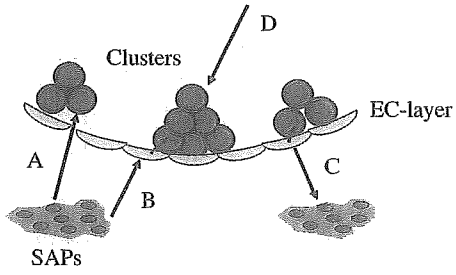

Figure 5. Model of HSC emergence in the P-Sp/AGM region. (**A**) Hematopoietic clusters are directly generated from SAPs. (**B**) Hematopoietic clusters are generated from SAPs via ECs as if SAPs were hemogenic endothelial progenitor cells/angioblasts. (**C**) Both hematopoietic clusters and SAPs are generated by hemogenic ECs. (**D**) Hematopoietic clusters have migrated from the other sources via circulation.

the clusters is very faint in the mouse embryo (Bertrand et al. 2005). Ac-LDL only is incorporated into macrophages and ECs. However, some embryonic cells might be able to incorporate Ac-LDL only in in vitro culture. A possible model is shown in Figure 5. Lineage-tracing experiments and further characterization of the cell nature will be necessary to understand the mechanisms how HSC generation is regulated in the mouse embryo.

◦ Concluding Remarks

We expect that these developmental approaches will enable us to understand the relationship between ECs and HCs for future clinical applications.

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症例報告

CD7/CD13陽性急性分類不能型白血病

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Acute Unclassified Leukemia with CD7 and CD13 Positivity

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Abstract We report a 14-year old boy with an acute unclassified leukemia (AUL) with CD7 and CD13 expression without other lineage-specific markers. He was diagnosed with T-cell acute lymphoblastic leukemia (ALL) at initial presentation because of the presence of a mediastinal mass and a negative cytochemical reaction for myeloperoxidase (MPO). He entered complete remission with standard chemotherapy for ALL and underwent allogeneic bone marrow transplantation (BMT) from an HLA-identical sibling at the 8th month. The post transplantation course was uneventful. The disease recurred in the bone marrow (BM) and in the skin 23 months after BMT. We characterized the blast cells again and made the diagnosis of AUL because of negative cytoplasmic expression for lineage-specific antigens: MPO, CD3 and CD79a. After unsuccessful induction treatment, he presented multiple bone metastases and hypercytokinemia-like symptoms. Finally, he entered hematological remission with cyclophosphamide and prednisolone 4 months after relapse. With a local irradiation to the right tibia, he received allogeneic peripheral blood stem cell transplantation (PBSCT) from the same donor as in the initial BMT. Chronic graft-versus-host disease in the oral mucosa and liver required intensive immunosuppressive therapy for 6 months and he relapsed in the BM 12 months after PBSCT. Although it is not well recognized in the literature, AUL with CD7 and CD13 positivity should be categorized as a unique entity with a dismal prognosis.

要　旨　発症時14歳の男児．表面マーカーはCD7，CD13のみ陽性で，光顕MPO陰性，縦隔腫瘤の存在よりT細胞性急性リンパ性白血病と診断した．第1寛解にてHLA一致弟より同種骨髄移植を施行したが，移植後23カ月時に皮下浸潤を伴い骨髄再発した．このとき，細胞質内CD3（cCD3），cCD79a，cMPOがすべて陰性と判明し，急性分類不能型白血病と診断を変更した．化学療法に反応せず，多発性骨転移および高サイトカイン血症様の病態を呈したが，cyclophosphamideとprednisoloneを投与後に骨髄および末梢血より芽球は消失した．右脛骨に対する局所放射線照射後，第1回移植と同一ドナーより末梢血幹細胞移植を施行した．第2回移植から12カ月後，慢性GVHDに対して用いていたFK506を減量中に骨髄第2再発をきたした．CD7/CD13陽性急性白血病は予後不良と考えられ，独立した疾患として扱う必要がある．

Key words: acute unclassified leukemia, CD7, CD13, allogeneic bone marrow transplantation, extramedullary involvement

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I. はじめに

白血病細胞の細胞系列は，形態の観察に加え，細胞化学染色および免疫学的検索に基づいて決定される．免疫学的検索は細胞系列のみならず，リンパ系芽球の成熟段階を特定するうえでも重要である[1]が，これらの検索に

ても細胞系列の特定について十分な所見が得られない例が少数ながらも存在し，WHO分類ではacute leukemia of ambiguous lineageとして一括されている[2]．そのなかで，細胞系列特異的マーカーを欠く一群は急性分類不能型白血病（AUL）として分類され，多能性幹細胞起源であると考えられているが[2]，詳細な病態はいまだに明らかではない．

今回われわれは，細胞系列特異性の高いマーカー[3-6]である細胞質内myeloperoxidase（cMPO），cCD3，cCD79aがいずれも陰性であったCD7/CD13陽性AULの1例を経験したので文献的考察を交えて報告する．

II. 症　　例

症例：初診時14歳，男児．

主訴：発熱．

既往歴：5カ月から12歳まで，てんかん（複雑部分発作）に対しvalproate内服していた．1歳時，川崎病（冠病変の合併はなし）．

家族歴：弟（3歳差）もてんかん歴あり（複雑部分発作；内服は行っていない）

現病歴：1999年9月（14歳時），発熱が持続したため近医を受診したところ，末梢血検査にて血球減少および芽球の出現を認めたため，急性白血病が疑われ，紹介入院となった．

初発時現症：身長161 cm，体重43 kg．ϕ5 mm前後の頸部リンパ節を両側とも1～2個ずつ触知する以外は，表在リンパ節腫脹，肝脾腫，皮疹を認めなかった．

初発時検査所見（Table 1A）：骨髄ではリンパ芽球様の芽球（Fig. 1A）の増多を認めたが，芽球表面抗原はCD7，CD13のみ陽性であった．染色体分析では複雑型核型異常を示し，コンセンサスプライマーを用いたpolymerase chain reaction（PCR）[7]にてT cell receptor（TCR）γおよびTCRδの再構成は認められなかった．髄液浸潤は認めなかったが，胸部X線および胸部CTにて縦隔腫瘤の形成を認めたため吸引細胞診を行ったところ，末梢血および骨髄で認められたものと同様の形態を示すリンパ芽球様細胞を認めた．

初発時経過：光顕的にMPO陰性であり，CD7が陽性で，縦隔腫瘤が存在することからT細胞性急性リンパ性白血病（ALL）と診断し，東京小児がん研究グループ

Table 1 Laboratory findings on initial admission (A) and at first relapse (B)

(A)			CD8	2.2 %
Peripheral blood		TCR rearrangement	Negative	CD10	1.4 %
WBC	3,100 /μl	Chromosome:		CD19	0.9 %
Blast	40.0 %	45～50, XY, +4, add (9) (q34), −11, +2～7 mar	14/20 cells	CD20	0.6 %
RBC	3.87×10⁶ /μl	46, XY	6/20 cells	CD13	92.4 %
Hb	10.9 g/dl			CD14	0.7 %
Ht	32.4 %	Surface antigens (CD45 gating; bone marrow)		CD33	3.4 %
Plt	99×10³ /μl	CD1	0.4 %	CD41	4.2 %
		CD2	2.5 %	GP-A	3.8 %
Bone marrow		CD3	1.6 %	CD34	0.8 %
NCC	34,000 /μl	CD4	0.5 %	CD56	0.3 %
Blast	86.5 %	CD5	26.2 %	HLA-DR	4.4 %
(MPO negative)		CD7	99.0 %	Aspiration cytology (mediastinum mass)	
MgK	3.1 /μl			Lymphoblastoid cells in medium size are seen.	
(B)		RT-PCR	MLL/AF4 (−), M-bcr/abl (−), m-bcr/abl (−)	Cytoplasmic and nuclear antigen (CD45 gating; bone marrow)	
Peripheral blood				MPO	0.6 %
WBC	1,300 /μl			cCD3	0.6 %
Blast	65.0 %			cCD79a	10.9 %
RBC	3.60×10⁶ /μl			TdT	14.5 %
Hb	11.3 g/dl	Chromosome:			
Ht	32.4 %	46, XY, del (2) (q11), t (4; 4) (q31 ; 33),der (8) t (2; 8) (q13; q22)	1/20 cell	Skin biopsy of the right pretibia	
Plt	42×10³ /μl	49, X, −Y, −7, add (9) (q34),−12, +6 mar	1/20 cell	Lymphoblastoid cells with CD7⁺ and CD13⁺ invaded diffusely from subcutaneous tissue to dermis.	
Bone marrow		46, XY	18/20 cell		
NCC	56,000 /μl			⁶⁷Ga scintigraphy	Negative
Blast	75.0 %				
(MPO negative)					
MgK	3.1 /μl				

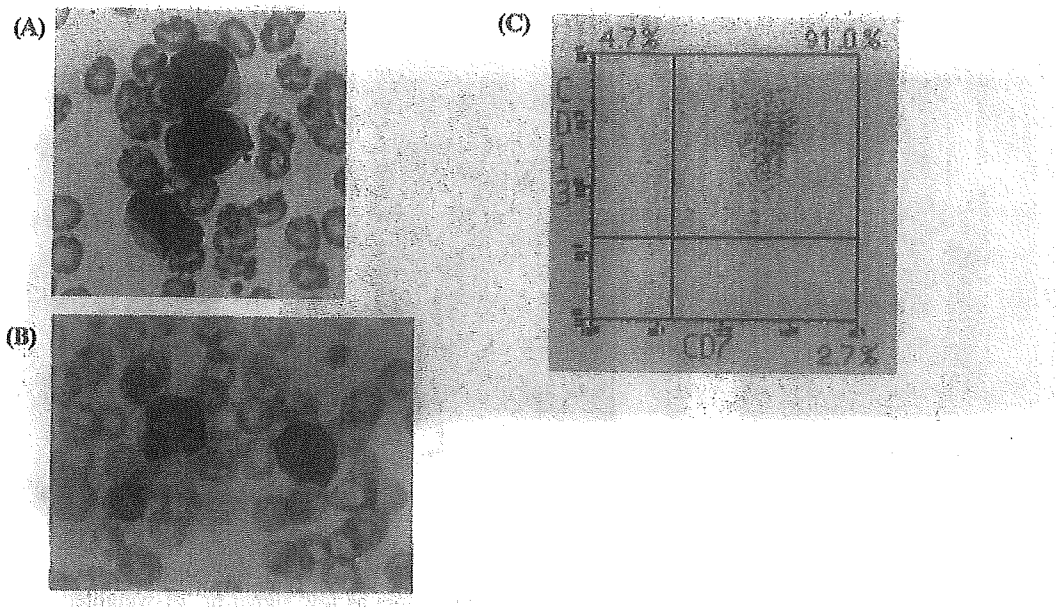

Fig. 1 Blasts in the bone marrow (BM) at initial diagnosis (A) and relapse (B), May-Giemsa stain, ×1,000, and the flow cytometric staining of blasts in the BM at relapse (C)
The morphological appearance of blast cells was lymphoblastoid (A, B), and blast cells were CD7/CD13 double positive (C).

(TCCSG) L99-15 HEX プロトコールにて化学療法を開始した．寛解導入療法開始後 43 日目に骨髄寛解を確認し，縦隔腫瘤も消失したが，寛解導入療法開始後 8 日目の芽球が 126 /μl と prednisolone (PSL) 初期反応性は良好ではなく，移植適応となった．2000 年 5 月に全身放射線照射 (TBI) 12 Gy, etoposide (VP-16) 60 mg/kg×1, cyclophosphamide (CPA) 60 mg/kg×2 を前処置として，HLA 一致弟から第 1 寛解にて同種骨髄移植 (BMT) を行った．移植片対宿主病(GVHD)予防は短期 methotrexate (MTX)＋cyclosporine (CyA) で行った．移植後経過は良好で，急性 GVHD は grade I (皮膚 stage 1，肝 stage 0，腸管 stage 0)，慢性 GVHD は認められなかった．

再発後経過：発症より 31 カ月後の 2002 年 4 月に，下肢の暗赤色の腫瘤形成および血球減少が出現した．末梢血，骨髄および腫瘤部の皮膚生検にて CD7/CD13 二重陽性の芽球 (Fig. 1B, C) を認めたことより，皮膚浸潤を伴った骨髄再発と診断した．このとき，細胞質内抗原の検索を行ったところ，cCD79a, cCD3, cMPO すべて陰性であったため (Table 1B)，AUL と診断した．TCCSG L99-15 HR プロトコールにて化学療法を開始したが寛解に到達せず，vincristine (VCR)，PSL，VP-16，cytosine arabinoside (Ara-C) により再度寛解導入を試みたが無効であり，多発性骨浸潤をきたした (両側脛骨近位，左上腕骨遠位，左仙腸関節部内側，右上腕骨遠位，右尺骨遠位；Fig. 2)．次いで，fludarabine，Ara-C，G-CSF による FLAG レジメンを行うも奏効せず，発熱，凝固線溶系異常，血小板減少，フェリチンおよび LDH の高値などの高サイトカイン血症様の病態を呈した．原病の急激な増悪と考え，PSL 30 mg と CPA 1.5 g を投与したところ速やかに解熱し，異常検査所見も正常化した．その後，骨髄および末梢血から芽球は消失したが，画像上骨病変が残存したため，もっとも画像所見の著明な右脛骨に局所放射線照射 (18Gy/9 分割) を行った後，2002 年 9 月 11 日に ifosfamide (IFM) 2 g/m²×4，melphalan (L-PAM) 90 mg/m²×2 を前処置として，第 1 回移植と同一のドナーからの末梢血幹細胞移植 (PBSCT) を行った．GVHD 予防は移植前から継続していた PSL に短期 MTX を併用した．急性 GVHD を認めなかったが，移植後 82 日目から口腔粘膜と肝臓の慢性 GVHD が出現し，PSL と FK506 の投与を要した．その後 PSL は中止とし，FK506 のみ継続していた．移植後も画像上は骨病変が残存していたが，2003 年 6 月に施行した右脛骨の骨生検では腫瘍細胞の浸潤は認められなかった．第 2 回移植から 12 カ月後の 2003 年 10 月 6 日に発熱と血球減少が出現し，末梢血および骨髄で CD7/CD13 陽性芽球を認め，骨髄第 2 再発と診断された．

III. 考　　察

CD7 は T 細胞分化段階の最も初期から発現するほか，骨髄系細胞の分化段階初期にも一過性に発現することが

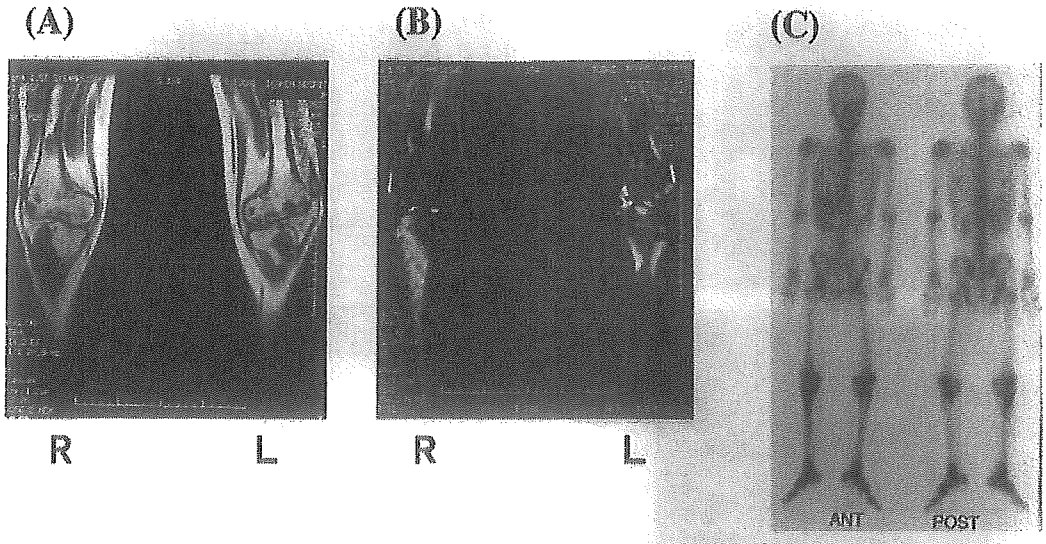

Fig. 2 MRI (A: T1-weighted, B: T2-weighted) and ^{67}Ga scintigraphy (C) findings of multiple bone metastases
Multiple bone metastases were detected in bilateral tibiae, fibulae and femora as low signal on T1-weighted (A) and high signal on T2-weighted (B). Abnormal ^{67}Ga uptakes were detected in right tibia, bilateral humeri, right ulna, and left sacroiliac joint.

知られている[8]．一方，CD13は骨髄系細胞の分化初期から発現する．したがってCD7/CD13陽性急性白血病はきわめて未分化な造血細胞が腫瘍化したものと推測される．本症例の芽球の表面抗原はCD7, CD13のみ陽性で，CD3, CD19, CD33, CD56は陰性であった．細胞系列特異性の高いcCD3，cCD79a，cMPO[3-6]がいずれも陰性であったことより，AULと最終的に診断した．AULの鑑別診断として，骨髄系抗原陽性ALL，リンパ系抗原陽性急性骨髄性白血病（AML）およびAML M0があげられる[2]．現行のAML M0の診断基準[9]ではリンパ系抗原の発現が弱い骨髄系抗原陽性ALLの鑑別が困難であることが指摘されており[10,11]，芽球の細胞系列決定が困難な症例に対しては，細胞表面抗原，TCR/Ig再構成，特殊染色などに加えて，細胞質内抗原の検索が必須である．

Bassanらは，CD7/CD13陽性白血病の6例の臨床症状および芽球の性状について報告している[12]．6例全例においてCD33やHLA-DRを同時に発現しており，本症例のようにCD7，CD13のみ陽性の症例はなかった．また，T細胞系列への分化を示唆するTCR再構成およびCD3 εmRNAの発現は，6例全例で陰性であった．吉田らは，縦隔腫瘤を伴ったCD7陽性AML M0症例を報告しているが[13]，その症例は光顕MPO陰性，表面抗原はCD7, CD13, CD33, CD34, HLA-DRが陽性で，cMPOおよびcCD3が陰性と，Bassanらが報告したCD7/CD13陽性白血病と同一であった．Bassanらは縦隔腫瘤陽性例を骨髄系抗原陽性T細胞性ALLとして除外していたが，吉田らの症例と本症例はともに縦隔腫瘤を形成しており，縦隔腫瘤はCD7/CD13陽性急性白血病において共通に認められる可能性がある．本症例で認められた皮膚，骨への髄外浸潤と腫瘍細胞の増殖に伴い出現した高サイトカイン血症様の病態については，Bassanら[12]および吉田[13]らの報告のほか，CD7陽性AML[14,15]やAML M0[10,11]にも記載がなく，本症例に特徴的な所見であった．Bassanらの報告では，治療を試みた5例中4例が治療抵抗性で，予後は不良であったが，1例のみmitoxantrone, Ara-C，VP-16とAML型の化学療法にて寛解に到達した後，大量Ara-C，TBIを前処置に自家BMTを施行され，報告時まで寛解が維持されていた[12]．吉田らの症例は，idarubicin＋Ara-Cを2コース施行した後，寛解を得た[13]．本症例は，初発時はALL型の寛解導入療法にて寛解に到達したが，第1再発後はALL型，AML型いずれの治療に対しても抵抗性を示した．欧米において再発・治療抵抗急性白血病に対し用いられ[16]，小児での有効性も報告されているFLAGレジメン[17-19]を試みたが，反応は認められなかった．その後，腫瘍細胞の急激な増加によるものと推測される全身状態の悪化を認めたため，緊急避難としてPSLとCPAを用いたところ，全身状態の改善とともに骨髄寛解が得られた．

最近，HLA一致同胞からのBMT後1年以内に再発した成人ALL2例に対し，初回移植と同一ドナーからのPBSCTと移植後早期の計画的ドナーリンパ球輸注（DLI）を施行し，長期寛解を得たとの報告があった[20]．2例とも全身型の慢性GVHDを認めており，骨髄における移植片対白血病（GVL）効果が示唆されるが，う

ち1例は髄外単独再発をきたしていた．免疫学的逃避が生じやすい髄外病変に対しては，十分なGVL効果は期待できないと考えられており[21]，骨生検で腫瘍細胞を認めなかったものの画像所見が残存していた本症例は，髄外病変から第2再発に至った可能性があると考えられた．

CD7/CD13急性白血病はこれまでの診断基準では見落とされていた可能性がある．本症例および過去の報告例は，特異な病態を呈し，予後不良であったため，今後はその生物学的特性や治療反応性を解明するために，独立した疾患として扱い，症例を蓄積する必要があると考えられる．

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症例報告

後天性サイトメガロウイルス感染症小児に合併した免疫学的血小板減少性紫斑病

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後天性サイトメガロウイルス感染症小児に合併した免疫学的血小板減少性紫斑病

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Immune Thrombocytopenic Purpura Associated with Acquired Cytomegalovirus Infection in Children

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Abstract Immune thrombocytopenic purpura (ITP) is an infrequent complication of acquired cytomegalovirus (CMV) infection. We describe two immunocompetent children with ITP, which was associated with CMV infection. We also reviewed reports or abstracts on 13 similarly affected children. Both patients had severe thrombocytopenia and mild increase of serum AST and ALT. Their bone marrow examinations were consistent with platelet consumption. They also had a high titer of anti-CMV IgM in serum. One of them responded to a conventional intravenous high-dose immunoglobulin therapy. The other patient, who failed to respond to the above therapy, was successfully treated with an intravenous high-dose of an anti-CMV high-titered immunoglobulin preparation. A reveiw of the literature showed the characteristics of ITP associated with CMV infection at diagnosis as follows: predominance in males; comparatively severe thrombocytopenia, but a mild bleeding tendency; mild lymphocytosis and mononucleosis in peripheral blood; mild elevation of serum AST and ALT; elevation of platelet associated-IgG in serum; normal or increased counts of magakaryocyte in bone marrow. Half of the patients underwent spontaneous remission or responded to conventional immunosuppressive therapy, but the remaining patients required a more aggressive immunosuppression or CMV-specific therapies. All patients eventually recovered and had no recurrence.

要 旨 血小板減少性紫斑病（ITP）を合併した後天性サイトメガロウイルス（CMV）感染症の自験例2例と文献報告例13例との臨床所見をまとめ，考察を行った．15症例の年齢は2カ月から8歳，性別では12例が男児であった．血小板数は1,000～33,000/μlで，重篤な出血症状はなかった．白血球数は正常から軽度上昇までで，リンパ球優位を5例，異型リンパ球を4例に認めた．PA-IgGは13例で上昇，骨髄巨核球は全例で増加し，AST，ALTは全例で上昇していた．治療は，2例が無治療，4例がプレドニゾロン（PSL），4例が大量ガンマグロブリン（IVIG）療法で軽快した．残る5例は初期治療に反応せず，1例がメチルプレドニゾロンパルス療法（pulse）で，2例がデキサメサゾンpulseで，1例がガンシクロビルで，1例がCMV高力価大量IVIGで血小板は正常化した．予後は，全例急性型で軽快し，慢性型への移行例や再発例はなかった．

Key words: immune thrombocytopenic purpura, acquired cytomegalovirus infection, intravenous immunoglobulin

I. 緒 言

後天性サイトメガロウイルス（CMV）感染症は，大部分の健常児では不顕性に終わる．時に発熱や肝脾腫を伴い，肝細胞障害と末梢血リンパ球や異型リンパ球が増加する単核球症を示すが，免疫学的血小板減少性紫斑病（ITP）を伴うものはまれである．

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今回われわれは，CMV感染症が誘因と考えられたITPの2症例を経験した．自験例に，本邦の小児における文献報告例13例を加え，小児の後天性CMV感染症によるITPの臨床像について考察を行ったので報告する．

II. 症　　例

1. 症　例　1

患児：1歳1カ月，男児．

主訴：点状出血斑と紫斑．

家族歴：特記すべきことはない．

既往歴：アトピー性皮膚炎で卵と乳製品の摂取制限を行っている．

現病歴：3日前に左前頭部の打撲部に紫斑が，2日前に四肢に点状出血斑と紫斑とが出現し来院した．発熱はなく全身状態は良好であった．

現症：四肢と前胸部に点状出血を，左前頭部，左腕，右大腿部，背部に紫斑を認めた．口腔粘膜に出血斑は認めない．肝脾は触知せず，リンパ節も触れなかった．

検査所見：血小板数の減少とリンパ球優位の白血球の増加，肝トランスアミナーゼの上昇を認めた．詳細はTable 1に示す．

臨床経過：末梢血血小板数の減少は，骨髄巨核球の増加と血小板関連IgG（PA-IgG）の増加からITPによるものと診断した．AST，ALTの上昇と異型リンパ球の増加，CMV IgM抗体の存在からCMV感染症が誘因と考えた．治療には，大量ガンマグロブリン（IVIG）療法（1 g/kg）とプレドニゾロン（PSL）の投与を行ったが，血小板数が20,000/μl前後で推移したため，CMV高力価大量IVIG（1 g/kg）を投与した．投与後，血小板数は速やかに正常化し，血小板減少の再発は認めない．発症9カ月後に，CMV IgMの消失を確認した（Fig. 1）．

2. 症　例　2

患児：1歳2カ月，女児．

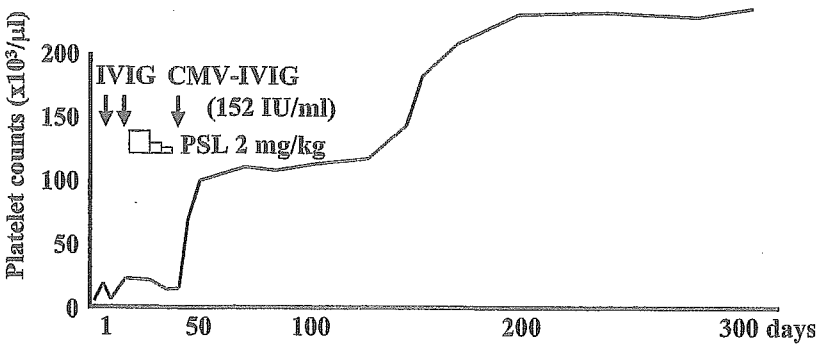
主　訴：点状出血斑と紫斑．

家族歴，既往歴：特記すべきことはない．

現病歴：6日前に両頬部に点状出血斑が，5日前から

Table 1　Laboratory findings (Patient 1)

WBC	15,700 /μl	Na	141 mEq/l	CMV IgM	8.41 (+)
Neutro	20.5 %	K	3.8 mEq/l	CMV IgG	11.4 (+)
Mono	6.5 %	Cl	108 mEq/l	C7HRP	(−)
Lymph	68.0 %	BUN	10 mg/dl	EBV EA-DR	<10
Aty.lym	3.5 %	Cr	0.2 mg/dl	EBV VCA IgM	<10
RBC	459×10⁴ /μl	TP	7.1 g/dl	EBV VCA IgG	<10
Hb	12 g/dl	AST	125 U/l	EBV EBNA	<10
Ht	35 %	ALT	151 U/l	IgG	683 mg/dl
Plt	1,000 /μl	LDH	502 U/l	IgA	22 mg/dl
APTT	35.0 sec	T-Bill	0.5 mg/dl	IgM	91 mg/dl
PT	99 %	CRP	0.14 mg/dl	PA-IgG	227.8 ng/10⁷ platelets
BM NCC	127,250 /μl			Megakaryocyte	69 /μl

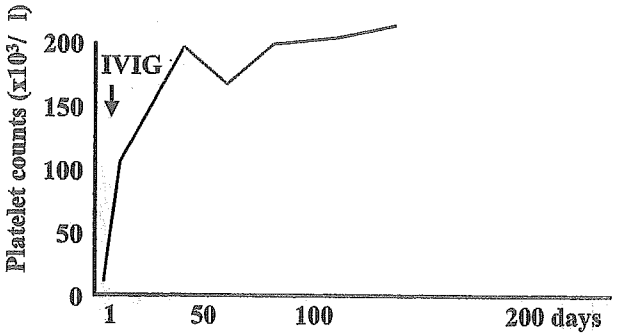


AST	125	69	39	36	45	26
ALT	151	114	27	16	36	13
CMV-IgM	8.41(+)	6.06(+)	4.00(+)	1.35(+)	0.59(-)	0.61(-)
CMV-IgG	11.4(+)	42.4(+)	42.9(+)	48.6(+)	52.6(+)	64.6(+)

Fig. 1　Clinical course (Patient 1)

Table 2 Laboratory findings (Patient 2)

WBC	8,000 /μl	Na	139 mEq/l	CMV IgM	3.96 (+)
Neutro	34.0 %	K	4.1 mEq/l	CMV IgG	27.8 (+)
Mono	2.0 %	Cl	103 mEq/l	C7HRP	N.D.
Lymph	63.0 %	BUN	9 mg/dl	EBV EA-DR	<10
Aty.lym	0.0 %	Cr	0.2 mg/dl	EBV VCA IgM	<10
RBC	452×10⁴ /μl	TP	6.4 g/dl	EBV VCA IgG	<10
Hb	13 g/dl	AST	45 U/l	EBV EBNA	<10
Ht	36 %	ALT	41 U/l	IgG	N.D.
Plt	7,000 /μl	LDH	319 U/l	IgA	N.D.
APTT	32.3 sec	T-Bill	0.4 mg/dl	IgM	N.D.
PT	108 %	CRP	0.13 mg/dl	PA-IgG	285.2 ng/10⁷ platelets
BM NCC	135,000 /μl			Megakaryocyte	194 /μl



AST	45	42	34	34
ALT	41	20	19	15
CMV-				
IgM	3.96(+)	2.68(+)	1.68(+)	
IgG	27.8(+)	38.4(+)	54.1(+)	

Fig. 2 Clinical course (Patient 2)

全身に点状出血斑と下肢に紫斑が出現した．発熱はなく全身状態は良好であった．

現症：顔面と四肢を中心に点状出血斑を，下肢に紫斑を認めた．口腔粘膜に出血斑は認めなかった．肝脾は触知せず，リンパ節も触れなかった．

検査所見：血小板数の減少と軽度の肝トランスアミナーゼの上昇を認めた．詳細は Table 2 に示す．

臨床経過：末梢血血小板数の減少は，骨髄巨核球の増加と PA-IgG の増加から ITP によるものと診断した．AST，ALT の上昇と CMV IgM 抗体の存在から CMV 感染症が誘因と考えた．通常の大量 IVIG 療法（1 g/kg）を行ったところ，血小板数は速やかに正常化した．その後も血小板数は正常範囲内にあり，血小板減少の再発は認めない．発症 1 カ月後に AST，ALT は正常化した．発症 4 カ月後の現在，CMV IgG は増加し，CMV IgM は減少してきているものの，IgM 抗体は完全には消失していない（Fig. 2）．

III. 自験例と文献例のまとめ

本邦における CMV 感染症に伴う ITP の小児報告例の臨床像をまとめて Table 3 に示す（先天性および免疫不全症に伴うものは除く）．発症年齢は，2 カ月～8 歳（中央値: 1 歳 2 カ月）で，性別は，12 例が男児であった．出血症状は，2 例で鼻出血や歯肉出血を認めた以外は全例皮膚出血のみであった．リンパ節腫大は 1/4 例で，肝脾腫は 6/10 例にみられた．発症時の血小板数は，1,000～33,000（中央値: 6,000）/μl であった．白血球数は 5,900～15,700/μl で，リンパ球増加を 5/9 例に，異型リンパ球増加を 4/9 例に認めた．AST，ALT は全例で軽度上昇していた．骨髄巨核球は，検索された全例で正常ないしは増加しており，PA-IgG は 9/11 例で増加していた．治療とそれに対する反応は，2 例が無治療で，4 例が PSL で，4 例が大量 IVIG 療法で軽快した．残る 5 例は初期治療への反応が悪く，最終的に，1/3 例がメチルプレドニゾロン（mPSL）パルス療法（pulse）で，2 例がデキサメサゾン（Dex）pulse で，1 例がガンシクロビル（GCV）で，1 例が CMV 高力価大量 IVIG 療法で，血小板数はそれぞれ正常化した．予後は，初期治療に抵抗した例も含めて全例急性型で軽快しており，慢性型への移行例や再発例はなかった．

IV. 考　　察

CMV 感染症に伴う ITP は男児に多く，軽度の肝細胞障害，末梢血リンパ球の増加や異型リンパ球の出現などの単核球症を伴い，診断時の血小板数が比較的低値を示すことを特徴としている．とくに血小板数は 11 例（73.3%）が 20,000/μl 未満で，そのうち 8 例（53.3%）が 10,000/μl 未満であった．出血症状は，乳幼児が多いためか皮膚出血のみが多いが，年長児では鼻出血や歯肉出血の粘膜出血症状が強い症例もあり注意を要する．血

Table 3 Characteristics of children with ITP associated with acquired CMV infection

	Age	Sex	Plt ($\times 10^4/\mu l$)	BM mega ($/\mu l$)	PA-IgG ($ng/10^7 plt$)	WBC ($/\mu l$)	Lymph atyp-L (%)	AST (U/l)	ALT (U/l)	CMV IgM	CMV IgG	Treatment	Outcome	Ref
1	2m	M	0.2	inc.	<12.5	12,700	87.5 0	86	54	+	NS	PSL	→GR	1
2	2m	M	1.1	inc.	262.2	10,100	82 1	54	22	+	NS	PSL	→GR	1
3	4m	M	2.3	175	22.3	133,300	72 0	60	66	+	+	PSL	→GR	2
4	5m	M	1.9	967.2	38.8	10,400	84 0	65	50	×20	×320	IVIG	→GR	3
5	5m	M	2.2	NS	NS	NS	NS	NS	NS	+	NS	PSL	→GR	4
6	1y	F	0.1	125	NS	9,520	NS	42	28	8.08	NS	PSL IVIG Dex pulse	→PR →PR →GR	5
7	1y7m	M	3.3	110	105.8	12,800	52 20.5	54	73	×160	×640	IVIG	→GR	6
8	1y10m	M	3.1	281	140.6	8,900	50 0	NS	NS	+	+	—	→GR	2
9	2y	M	0.1	inc.	598.5	5,900	NS	54	24	×80	NS	PSL IVIG mPSL pulse Dex pulse	→NR →NR →PR →GR	5
10	4y	M	1.5	NS	NS	NS	NS	NS	NS	+	NS	—	→GR	4
11	4y4m	F	0.5	NS	NS	NS	NS	inc.	inc.	NS	×156	PSL+IVIG	→GR	7
12	6y	M	0.5	inc.	—	NS	NS	199	197	5.44	15.0	IVIG mPSL pulse	→NR →GR	8
13	8y	M	0.1	187.5	1.529	6,800	38 8	62	72	+	NS	PSL IVIG mPSL pulse VCR+CyA GCV	→NR →NR →PR →NR →GR	9
14	1y1m	M	0.1	69	227.8	15,700	68 3.5	125	151	8.41	11.4	IVIG PSL CMV IVIG	→PR →NR →GR	pt.1
15	1y2m	F	0.6	194	285.2	8,000	63 0	45	41	3.96	27.8	IVIG	→GR	pt.2

CMV: cytomegalovirus, ITP: immunological thrombocytopenic purpura, Plt: platelet, BM: bone marrow, mega: megakaryocyte, WBC: white blood cell, lymph: lymphocyte, atyp-L: atypical lymphocyte, ref: reference, pt: patient, y: year, m: month, M: male, F: female, inc: increased, NS: not shown, GR: good response, PR: partial response, NR: no response, PSL: predonisolone, IVIG: intravenous high-dose immunoglobulin, Dex: dexamethasone, mPSL: methylpredonisolone, VCR: vincristine, CyA: cyclosporin, GCV: ganciclovir, −: negative, +: positive.

小板減少の機序に関しては，多くの症例で骨髄巨核球や PA-IgG が増加しており，通常の ITP と同じく免疫学的機序による血小板の破壊亢進が考えられる．

治療に関しては，粘膜出血等の出血症状の強いものは多くないものの，血小板数が低く年少児が多いため，2 例を除いて初期から ITP の治療が行われている．ITP の治療に対する反応は，2/3 の症例では良好な反応がみられたが，残り 1/3 の症例は，初期治療に反応せず，一部では出血症状も強いため，mPSL あるいは Dex pulse 療法など，より強い免疫抑制療法や GCV などの CMV 感染治療が必要であった．成人では，ITP に対する種々の治療に抵抗性で，最終的に GCV や CMV 高力価 IVIG が有効であった症例の報告も散見される[10-13]．小児でも通常の ITP 治療に反応しない場合は，早期から CMV 感染治療を併せて行うことを念頭に置くべきであろう．自験の症例 1 では，CMV 高力価大量 IVIG 療法が有効であった．急性期に通常の大量 IVIG 療法で血小板の増加が得られない場合には，CMV 高力価大量 IVIG 療法が治療選択肢の 1 つになりえると考えた．急性期に適切に治療すれば慢性型や再発例に移行することはまれであり，急性期の治療選択が重要である．

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血液の事典

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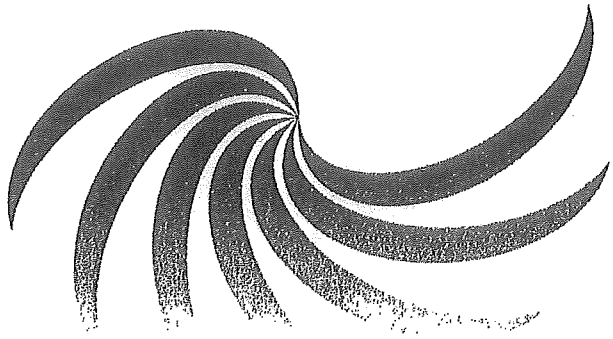

朝倉書店

巨核球は洞外膜に接して局在し，内皮有窓部からその細胞質突起が洞内に突出し，血小板を血中に放出する．赤芽球系細胞は時折，赤芽球島（erythroblastic island）を形成し，洞壁近くに位置する．この構造物は中心にマクロファージがあり，このマクロファージをcentral macrophageと呼んでいる．赤血球系細胞は洞壁通過時に脱核する．

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19 造血幹細胞

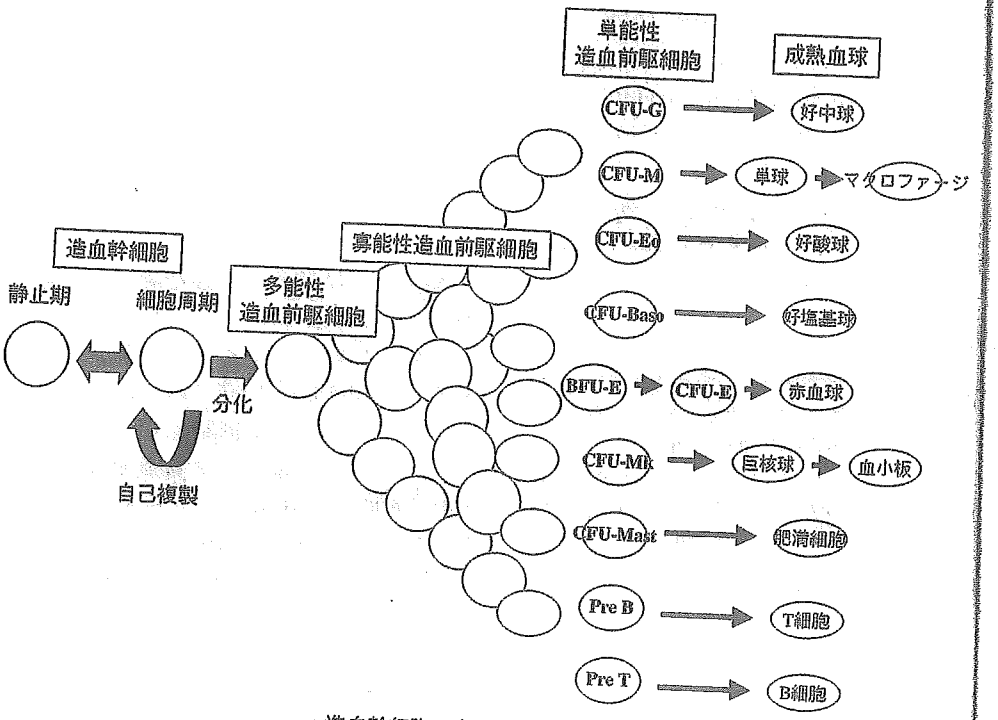
辻浩一郎

血液中には形態と機能を異にする種々の血球が存在するが，それらはいずれも固有の寿命で崩壊している．この膨大な数の血球を一生の間供給し続けるためには，血球の源となる未分化な細胞のプールが必要であり，これらの細胞を造血幹細胞と呼ぶ．造血幹細胞は，細胞分裂により自己と同じ能力を有する細胞を複製する能力（自己複製能）と，すべての成熟血球を産生する能力（多分化能）という二つの能力をあわせもつことにより，われわれの一生にわたる造血を枯渇させることなく維持しており，造血幹細胞移植においては，レシピエントの体内，主には骨髄において新たな造血を再構築し，長期にわたり維持することを可能としている．こうした造血幹細胞の能力は「長期造血再構築能」と呼ばれる．

■造血幹細胞の動態

図は，造血幹細胞から成熟血球が産生される過程を示している．恒常状態では多くの造血幹細胞は静止期にあり，必要に応じて細胞周期に入り細胞分裂する[1]．造血幹細胞は細胞分裂すると，その娘細胞は自己複製して再び造血幹細胞となるか，あるいは分化して多能性造血前駆細胞となる．多能性造血前駆細胞はすでに分化することが運命づけられた細胞で，多分化能は有しているが自己複製能はもたないことより，造血幹細胞とは区別される．

造血幹細胞由来の多能性造血前駆細胞は，細胞分裂を繰り返しながら次第にその多分化能を失い，数種類の血球系への分化能のみを有する寡能性造血前駆細胞を経



造血幹細胞の自己複製と分化

CFU-G：granulocyte-colony forming unit, CFU-M：macrophage-CFU, CFU-Eo：eosinophil-CFU, CFU-Baso：basophil-CFU, BFU-E：erythroid-burst forming unit, CFU-E：erythroid-CFU, CFU-Mk：megakaryocyte-CFU, CFU-Mast：mast cell-CFU, Pre-B：B cell precursor, Pre-T：T cell precursor.

て，単一の血球系への分化を運命づけられた単能性造血前駆細胞となり，最終的にはリンパ球を含むすべての成熟血球を産生する[2]．

■造血幹細胞の評価法

従来造血幹細胞の評価法としては，コロニー形成法，LTC-IC（long-term culture-initiating cell）測定法などが多く用いられてきたが，こうした*in vitro*での評価法はあくまで造血前駆細胞の評価法であって，長期造血再構築能を正しく評価しているわけではなく，その意味では造血幹細胞の評価法の代替法にすぎない．少なくとも現時点で信頼できる長期造血再構築能の評価法としては移植系以外にはなく，ヒトの場合はNOD/SCID（nonobese diabetic/severe combined immunodeficiency）マウスをレシピエントとする移植系がよく用いられる．NOD/SCIDマウスは，成熟リンパ球の欠損，マクロファージ活性の低下，補体活性の低下，NK細胞活性の低下などの特徴を有し，ヒトサイトカインの投与などの処置を必要とせず，ヒト造血幹細胞が安定して生着する．NOD/SCIDマウス，あるいはSCIDマウスの骨髄に生着可能なヒト細胞は，SRC（SCID mouse-repopulating cell）と呼ばれ，造血幹細胞に相当する細胞と考えられている．

■造血幹細胞の細胞表面形質

1）マウス造血幹細胞の細胞表面形質

成体マウス造血幹細胞には，単球/マクロファージ，顆粒球，B細胞，T細胞，赤芽球の分化抗原であるMac-1, Gr-1, B220, CD4, CD8, TER119などは発現されておら