Anatomical Society Symposium on extramedullary haematopoiesis

A symposium on extramedullary haematopoiesis was held on 11–12 September 1996 at the University of St Andrews. The following are abstracts of papers and posters presented at the meeting.

1 Emergence of intraembryonic haematopoietic stem cells in the avian and mouse embryo. By F. Dieterlen-Lievre, L. Pardanaud, I. Godin and A. Cumano* (introduced by D. B. Thomas). *Institut d'Embryologie Cellulaire et Moléculaire du CNRS et du Collège de France and * Institut Pasteur, Unité de Biologie Moléculaire du Gène, Paris*

Early in development haematopoietic stem cells (HSC) seed the blood-forming organ rudiments, constituting a self renewable reserve. Some years ago using an avian chimera model (quail embryo grafted on chick yolk sac), we established that these HSC come from the body of the embryo. We were able to ascribe the emergence of HSC to the region of the aorta. The existence of intraembryonic HSC is now being demonstrated in the mouse embryo by means of a 2 step cell culture method. We first showed the presence of progenitors in l0–25 somite embryos (8.5–l0 dpc) in the 'paraaortic splanchnopleura' (Godin et al. *Proc. Nat. Acad. Sci. USA* **92**, 1995). We now trace back the origin of these cells to the presomite and early somite embryo at 7.5 dpc, i.e. before blood circulation. Interestingly, the yolk sac produces progenitors that are short lived and do not yield lymphocytes. In contrast, the splanchnopleura produces multipotential progenitors that renew for at least 3 wk in vitro. The developmental relationship between the endothelial and haematopoietic lineages is studied in the avian model. We previously found that the splanchnopleural layer of the mesoderm produces endothelial cells (EC), while the somatopleural layer is colonised by extrinsic precursors. We now show that these precursors come from the somites and that 2 distinct endothelial lineages vascularise the embryo, a purely endothelial somitic or dorsal lineage; a splanchnopleural or ventral one endowed with a dual, endothelial and haematopoietic potential (Pardanaud et al. *Development* **122**, 1996).

2 The roots of the haematopoietic tree: finite ontogeny. By I. N. Rich (introduced by D. B. Thomas). *Division of Transplantation Medicine, Center for Cancer Research and Treatment, Richland Memorial Hospital, Columbia, South Carolina, USA*

The haematopoietic system can be considered similar to a tree, with the leaves representing the mature cells, the branches the progenitor cell populations and the trunk the stem cell compartment. But what part of the haematopoietic system can be compared with the roots of the tree? The answer is none, because the roots of the haematopoietic tree are nonhaematopoietic in nature. These are the cells that actually initiate haematopoiesis in the different organs and tissues during ontogeny. They are specific cells present in the embryonic epiblast and whose direct descendants have to undergo allocation into the allantois, extraembryonic yolk sac mesoderm, blood islands and part of the embryonic mesoderm. These specific cells are first observed as an alkaline phosphatase-positive (AP^+) population at the base of the allantois in late d 6 mouse embryos, but also express stage-specific embryonic antigen 1 (SSEA-1) on their surface. The AP^+ cells are destined to become germ cells in the gonads and are therefore called primordial germ cells (PGC). We have shown that PGCs can be cultured, expanded in vitro and induced to differentiate into haematopoietic cells in the presence of growth factors. During this induction, not only multipotential stem cells, but also the most primitive haematopoietic stem cell population in vitro, the cobblestone-area-forming cells (CAFC) can be detected. Primordial germ cells expanded on Matrigel in perfusion chambers in the presence of SCF, LIF, IL-3 and bFGF were transferred cell by cell to individual wells of a microtitre plate and cultured in the presence of different haematopoietic growth factor combinations. Early erythroid progenitors (BFU-E), myelomonocytic progenitors (GM-CFC), multipotential stem cells and PGCs could be produced. Preliminary transplantation experiments indicate that in vitro expanded PGCs can reconstitute lethally irradiated recipients. These results imply that the haematopoietic system can be initiated from a nonhaematopoietic stem cell population. It is postulated that this population is responsible for the sequential initiation of haematopoiesis during ontogeny and implies that a finite population of stem cells is present in each location, thereby possibly contributing to the time a particular organ or tissue remains haematopoietic during ontogeny. Since the bone marrow is the final stage of haematopoietic ontogeny under normal conditions, loss of this nonhaematopoietic stem cell population or failure to induce any quiescent cells into haematopoiesis means ultimate exhaustion of the haematopoietic system.

3 The AGM region initiates definitive haematopoiesis in the mouse embryo. By A. Medvinsky and E. Dzierzak (introduced by D. B. Thomas). *National Institute for Medical Research, London*

The adult haematopoietic system of mammals is a dynamic hierarchy of cells with the haematopoietic stem cell at its foundation. During embryonic development, the source and expansion potential of this cell remains unclear. The yolk sac is the first site of haematopoietic activity in the embryo. However, its haematopoietic hierarchy at early stages lacks CFU-S and longterm reconstituting haematopoietic stem cells (LTR-HSC) suggesting that it originally serves the immediate needs of the embryo. Recently an intraembryonic site of haematopoietic activity—the aorta-gonad-meso-

nephric (AGM) region—has been identified, which starts generating CFU-S progenitors in parallel to the yolk sac. To reveal the true source of this haematopoietic activity and LTR-HSCs, we undertook an experimental approach to prevent possible cellular interchange between early embryonic tissues. A novel in vitro organ culture system was developed in which early embryonic tissues were cultured in isolation. Under these conditions the AGM region is able to amplify significantly the number of CFU-S in contrast to the yolk sac. We also demonstrate that LTR-HSCs initiate autonomously and exclusively within d 10 AGM region. Furthermore, we observe a 15 fold in vitro enhancement in LTR-HSC within d 10 AGM region. The LTR-HSC generation occurs in the anterior part of the AGM region, whereas CFU-S are produced in both the anterior and posterior parts of the AGM region. These results are the first demonstration of expansion of LTR-HSC activity in vitro. The unique temporal and spatial properties of the embryonic AGM region should lead to a better understanding of the factors necessary for initiation and expansion of the definitive haematopoietic stem cell.

4 The origin of haematopoietic progenitors in the human embryo. By E. KELEMEN (introduced by D. B. THOMAS). *National Institute of Haematology, Budapest, Hungary*

Two types of haematopoiesis can be recognised during human development: primitive, and definitive. Until recently, most experts believed that extraembryonic blood cells are the ancestors even for definitive, intraembryonic haematopoiesis, and the role of stem cell migration was widely accepted, both for haemic cells and for germ cells. The fact that even early, primitive, embryonic haematopoiesis originates locally, cannot be questioned. All types of embryonic erythroblasts can be demonstrated even in yolk sac sinusoids, both at intraembryonic, and at extraembryonic (e.g., chorionic, allantoic) sites, before the start of circulatory fusion between extra- and intraembryonic vessels, i.e. by the end of the 3rd wk of development. However, this simple statement is not valid for definitive, fetal haematopoiesis. Animal studies from a number of laboratories suggest that stem cells for definitive haematopoiesis arise independently from extraembryonic sources, inside the embryo. In fact, intraembryonic stem cells have been identified as dense clusters developing in close association with the abdominal, ventral aortic endothelium, at first on its intravascular aspect, and later in the surrounding paraaortic mesenchymal tissue: the splanchnopleural mesoderm. Recently, it has been suggested that the stem cells are of similar origin in man. However, it is difficult to prove this conclusively because the extraembryonic and intraembryonic circulatory pathways fuse before the onset of aortic/paraaortic stem cell clusters. Thus, it is possible that aortic/paraaortic stem cells may originate from migrating extraembryonic stem cells. Furthermore, potentialities of yolk sac stem cells in animals are higher than one could suppose on the basis of previous work. Yolk sac haematopoietic stem cells of mice have greater reproductive capability than stem cells obtained from fetal liver, umbilical cord blood, or adult bone marrow. They are able to reconstitute adult immunocompromised animals even when introduced in very small numbers, e.g. 100 cells/ mouse. In addition, primordial germ cells appear to disturb the scene, because at the time when aortic/ paraaortic stem cell clones appear, migrating germ cells are already located at the very same splanchnopleural area, and are also able to initiate multilineage haematopoiesis.

5 Hepatic haematopoiesis. By D. B. THOMAS. *School of Biological and Medical Sciences, University of St Andrews*

Before the development of adequate medullary cavities, the liver of the mammalian fetus contains numerous blood cell precursors at various stages of maturation, including haematopoietic stem cells (HSC) which appear to be derived from intrasomatic precursors independently of HSC in the wall of the yolk sac. Following transplantation to allogeneic recipients, these cells are capable of establishing multilineage haematopoiesis, without generating the graft-versus-host reactions which complicate the transplantation of allogeneic bone marrow cells. While the fetal liver is the principal site of erythropoiesis, the virtual absence of differentiating granulocytes and lymphocytes from the liver, cannot therefore be due to the limited differentiation potential of hepatic HSC. Instead it must be attributed to the inability of the haematopoietic microenvironment (HME) accommodated by the liver to sustain the production of granulocytes or lymphocytes, which can be sustained in the bone marrow when medullary and hepatic haematopoiesis coexist. As development proceeds, haematopoiesis is transferred from the transient hepatic HME to the bone marrow. In the murine fetus, this transfer accompanies a reduction in the ability of the liver to establish stromal layers capable of sustaining haematopoiesis in vitro; a relative decrease in the adhesion of early blood cell precursors to stromal layers derived from the liver and the absence of a proliferative response to a decrease in the population size of early blood cell precursors in the liver. These observations are compatible with a progressive deterioration in the HME accommodated by the liver. The feasibility of retarding or reversing this ontogenetic deterioration has been evaluated in sudden, unexpected, infant deaths, in which persistent hepatic haematopoiesis has been accepted as a putative marker of chronic hypoxia; in osteopetrotic mice, in which development of the medullary cavities is defective and in myelofibrosis in which the space available for intramedullary haematopoiesis is reduced. Islands of blood cell precursors sometimes persist in the sudden infant death syndrome (SIDS)—and are probably correctly interpreted as markers of chronic hypoxaemia—but the liver does not appear to retain or to restore its ability to accommodate effective compensatory haematopoiesis in any of the conditions which have been studied. This is consistent with the limited haematopoietic support capacity of the liver described following destruction of the bone marrow by radioactive strontium. Intrasinusoidal blood cell precursors, which are probably derived from the bone marrow, may be conspicuous in SIDS and in myelofibrosis, but clearly do not reflect the retention or restoration of a fetal activity in which the differentiating erythroblasts are extravascular.

6 Regulation of the proliferation of haematopoietic progenitor and stem cells in fetal liver. By A. C. Riches. *School of Biological and Medical Sciences, University of St Andrews*

Cell proliferation in the haematopoietic system is regulated by a series of interacting feedback loops operating at different levels in this hierarchical cell renewal system. Different regulators act on the committed progenitor cells compared with the haematopoietic stem cells, where multiple signals often operate. Haematopoietic stem cells are normally in a quiescent state in medullary sites in the adult, whereas following perturbation of the haematopoietic stem cell pool, the surviving cells are rapidly recruited into cell cycle. These proliferation changes are regulated by locally produced factors which control the proportion of haematopoietic stem cells in cell cycle. A small peptide (AcSDKP) also has been demonstrated to exhibit an indirect regulatory activity on haematopoietic stem cells. This peptide may be derived, under physiological conditions, by enzymatic cleavage from thymosin b4. In murine fetal liver, the haematopoietic stem cells are in cell cycle. A regulator which switches quiescent haematopoietic stem cells into cell cycle is present. As the number of haematopoietic stem cells in fetal liver rapidly declines by birth then the production of this regulator in fetal liver also ceases. A similar regulatory activity is also detected in human fetal liver and fetal bone marrow during the hepatic phase of haematopoiesis but is not detectable in fetal thymus. The kinetics of the committed progenitor cells (GM-CFC) in human fetal liver changes markedly during gestation. At early stages (11–14 wk) the cells are quiescent whereas at later stages ($>$ 14 wk) the cells are proliferating. These changes correlate with the presence of inhibitory and stimulatory activities which modify the proliferation of GM-CFC. Thus, in the hepatic phase of haematopoiesis in the fetus, marked changes in the kinetic state of progenitors and stem cells are taking place associated with locally produced regulatory activities.

7 Haematopoiesis in the thymus. By M. D. Kendall, B. S. Mitchell* and U. Schumacher**. *Thymus Laboratory, Babraham Institute, Cambridge, *Anglo-European College of Chiropractic, Bournemouth, and **Human Morphology, University of Southampton*

The thymus, the major organ for T cell development, also acts as a site for other forms of haematopoiesis in all vertebrates (reviewed in Kendall, *Dev. Immunol.* **4**, 1995). This activity is prominent in embryos, but it is not generally appreciated how common haematopoiesis is in the adult thymus. For haematopoiesis to occur, the thymus must host the appropriate stem cells, and have a favourable microenvironment. Haematopoietic stem cells initially derive from the yolk sac, then from the liver, and finally from the bone marrow. The stem cells entering the embryonic thymus are multipotential, although in adult life many primitive cells are already lineage-restricted. The extent to which adult haematopoiesis in the thymus depends on the stimulation of dormant stem cells is not clear, since new stem cells could also enter from the circulation. There is good evidence for the cytokine microenvironment being favourable for haematopoiesis. Interleukin-3 (IL-3), the multipotential cytokine that stimulates proliferation and differentiation of pluripotent haematopoietic stem cells as well as lineage committed precursors of all myeloid cells, is produced by thymic epithelium. So too are other cytokines required as IL-3 synergists (IL-6, IL-1, *c-kit* ligand, and granulocyte stem cell factor-1 or G-SCF-1). Although IL-1a and G-SCF-1 are poorly produced by thymic cortical epithelial cells, stimulation of cultured thymic epithelial cells causes strong upregulation in the production of these cytokines. Of potential importance for the study of these events is the occurrence of myelopoeisis in the thymus of adult SCID/SCID mice. These mutants lack T and B cells (although some 'leaky' mice do have small numbers of both), and have a simple thymus of relatively undifferentiated epithelium (similar to embryonic and cultured thymic epithelial cells). There is no development of cortex or medulla, but normal histology and thymopoiesis is restored with thymocyte precursor reconstitution. This ultrastructural study of 8 adult male 3–4 mo old SCID/SCID mice (anaesthetised with ether and killed under Schedule 1) shows that the thymus contains immature stages of granulocytes, mast cells and plasma cells. Erythropoiesis was not observed in these mice, although thymic erythropoiesis is well documented for many vertebrates (Kendall & Ward, *Nature* **249**, 1974; Kendall, *Dev. Comp. Immunol.* **4**, 1980). Since the SCID mouse is used as an immunological model for thymus reconstitution and for creating 'organoid' cultures, the myelopoietic capabilities of the thymus of these mice needs to be considered.

8 Extramedullary haematopoiesis in vitro: blood from embryonic stem cells. By J. Ansell (introduced by D. B. Thomas). *ICAPB, Ashworth Laboratories, University of Edinburgh*

Mouse embryonic stem (ES) cells when cultured in vitro as aggregates differentiate into embryoid bodies (EBs) and subsequently can develop haematopoietic structures which contain progenitor cells for all haematopoietic lineages. We have defined the time point during such differentiation when EBs contain a small number of haematopoietic 'stem' cells that can contribute to the long-term rescue of lethally irradiated recipients. Although descendants of these EB-derived haematopoietic cells can contribute to both myeloid and lymphoid compartments of the haematopoietic system the proportion of these in the periphery is low. Recent data demonstrate that EB derived 'stem cells' in the spleen and marrow of irradiated recipients are however abundant, dormant and long-lived and will contribute more effectively to haematopoiesis after secondary transfer. The differentiation of haematopoietic elements in EBs can be enhanced by dimethyl sulphoxide (DMSO) and/or defined cytokine regimens and inhibited by retinoic acid. Using this system the molecular/cellular mechanisms involved in early commitment and differentiation of haematopoietic lineages are being studied by a variety of different methodologies including the use of subtractive DNA libraries, quantitation of protein expression by 2D gel electrophoresis and gene expression in EBs in situ.

https://doi.org/10.1046/j.1469-7580.1997.19020311.x Published online by Cambridge University Press

9 Analysis of haematopoietic gene expression in differentiating embryonal stem cells. By G. Graham, K. Ryan, I. Hayes and N. Hole* (introduced by D. B. Thomas). *Beatson Institute for Cancer Research, Glasgow and* * *Department of Zoology, University of Edinburgh*

The difficulties involved in analysing the gene expression that is important for the generation of haematopoietic stem cells in developing embryos has prompted many researchers to turn to the embryonal stem (ES) cell system as a model for developmental haematopoiesis. We have now mapped the time course of emergence of haematopoietic stem cells (HSCs) in developing ES cells and have demonstrated that both long term repopulating and transient engrafting stem cells are detectable at d4 and d5 after differentiation initiation (DI). Given the absence of detectable haematopoiesis at d3 post DI, it is likely that much of the molecular machinery required for the de novo generation of these HSCs is coming into play over this narrow 2 d time frame. We have therefore initiated subtractive hybridisation studies to analyse genes, levels of which are altered in concert with the generation of haematopoietic stem cells in the in vitro ES cell system, the hope being that this may provide unprecedented access to novel genes of importance in haematopoietic stem cell generation and function. The functional equivalence of the ES-derived HSCs with adult HSCs makes it likely that what we identify in our subtracted libraries may well shed much needed light on the regulation of adult as well as embryonal HSC function and generation. We have analysed a number of clones from the subtracted libraries and present descriptions of them. Thus far, around 70% of identifiable sequences isolated appear to be haematopoietic in origin and function. This suggests that the d3-d5 time frame during ES cell differentiation represents a rich source of both known and novel haematopoietic sequences, analysis of which is likely to be of value in enhancing our ability to understand HSCs and to manipulate them therapeutically.

10 Strategies to study haematopoiesis using embryonic stem cells. By L. Forrester (introduced by D. B.Thomas). *Centre for Genome Research, University of Edinburgh*

A major goal in haematopoietic research is to understand the molecular basis of haematopoietic stem cell development, proliferation and differentiation. Several gene products have been identified that clearly play a role in these processes. However, there is a great need to identify more novel genes and to assess their function specifically in haematopoietic lineages. We are using a gene trapping approach in embryonic stem (ES) cells as a route to identify such novel genes. Gene trap vectors consist of a reporter gene (*lac*Z) linked to a splice acceptor site. After integration into the intron of an active gene the splice acceptor site is used in the predicted manner resulting in the generation of a fusion transcript (and protein) between the endogenous trapped gene and reporter. The reporter gene expression is then under the regulatory control of the promoters and enhancers of the trapped gene and thus mimics its expression. A vast number of ES cell lines with different gene trap integrations can be generated in vitro and subsequently analysed with respect to expression and function both in vitro and in vivo. For example, ES cells can differentiate into a variety of haematopoietic cell lineages, including haematopoietic precursors, when placed under specific culture conditions. Thus by differentiating gene trap ES cell lines in vitro and subsequently staining for reporter gene expression it is possible to screen for those integrations that are expressed in haematopoietic lineages. ES cells can contribute to all cells, including the germline, of the developing embryo when injected into host blastocysts. As the gene trap integration is likely to mutate the endogenous trapped gene, its function in vivo can be assessed by transmitting the disruptive integration through the germline and breeding to homozygosity. The disruption of several haematopoietic-related genes have been shown to affect early embryonic development and it has proven impossible to assess the function of the mutated gene in haematopoietic development by conventional methods. We have developed a strategy to overcome this by aggregating ES cells with tetraploid embryos and using the fetal liver of these completely ES derived embryos to reconstitute the haematopoietic system of lethally irradiated mice. A combination of gene trapping and tetraploid embryo generation provides a strategy to functionally analyse genes that play a role in early haematopoietic development.

11 Towards clinical cord blood banking in the UK. By J. Hows, C. Donaldson, A. Nicol, P. Denning-Kendall, M. Nieda, H. Horsley and B. Bradley (introduced by D. B. Thomas). *Department of Transplantation Sciences, University of Bristol*

Allogeneic stem cell transplantation is limited by the need for close HLA matching of donor and recipient and therefore by donor availability. We are investigating umbilical cord blood (CB) as an alternative source of haematopoietic stem cells for patients in whom allogeneic bone marrow transplantation (BMT) is the treatment of choice but who lack a HLA identical sibling. A cord blood banking group (UKCBB) has been established by investigators in Belfast, Birmingham, Bristol, Edinburgh, London and Newcastle. The group is setting up a UKCBB network linked to CB banks and unrelated marrow donor registries world-wide. The ultimate aim of the group is to facilitate a pilot study to evaluate CB transplantation in comparison with unrelated marrow donor BMT. The main problem addressed so far in Bristol is the limited number of nucleated blood cells present in CB donations, about 10% of those present in the average donation for a larger adult recipient. From 97 consecutive CB donations the mean total volume was 115 ml (s.d. $\pm$37), and total nucleated cell count 14.8×10^8, (s.d. $\pm$10.3). We chose to study the stem cell potential of CB using long-term haematopoietic cultures (LTC), because long-term culture initiating cells (LTC-IC) have 'stem' cell properties of high proliferative potential and self renewal. We have shown that the frequency of LTC-IC in CB blood is not significantly higher than in normal marrow, however individual CB LTC-IC have higher proliferative and self renewal capacity than NBM LTC-IC (normal adult bone marrow LTC-IC). It is possible to expand numbers of CB LTC-IC in vitro but it is not yet certain whether this is safe, necessary or practical for extending CB transplants to adult recipients. Volume reduction by red cell depletion will enhance cost effectivity

https://doi.org/10.1046/j.1469-7580.1997.19020311.x Published online by Cambridge University Press

of CB banking. Despite early reports to the contrary, it is possible to red cell deplete cord blood donations, using simple sedimentation techniques with acceptable nucleated cell recovery. We have found average recoveries of 77% nucleated cells, 92% clonogenic cells, and 86% CD34+ cells using 3% gelatin as the sedimentation agent. Others have shown excellent recovery of buffy coat cells using the Baxter Optipress system and hydroxy-ethyl starch sedimentation and centrifugation. Using controlled rate freezing we have shown that optimal recovery of CD34+ cells was attained using freezing at −1 °C per min, DNA-ase to prevent cell clumping, and 5–10% DMSO. The addition of hydroxy-ethyl starch as an extracellular cryoprotectant did not improve recovery. Using paired LTC experiments no decrease in function of LTC-IC was detected after cryopreservation, although mature progenitor cells measured by clonogenic assays were significantly reduced by cryopreservation. In conclusion, in vitro studies have provided evidence that CB haematopoietic stem cells have immense potential for proliferation and self renewal, and can be successfully manipulated in vitro, making cord blood banks an interesting alternative to volunteer marrow donor registries.

12 Myeloid metaplasia: clonal extramedullary haematopoiesis. By D. T. Bowen (introduced by D. B. Thomas). *Department of Molecular and Cellular Pathology, Ninewells Hospital and Medical School, University of Dundee.*

Agnogenic myeloid metaplasia (synonym: myelofibrosis) is a myeloproliferative clonal haematological malignancy characterised by bone marrow fibrosis, extramedullary haematopoiesis and an increased leukaemic transformation risk. Principally a disease of the elderly, clinical presentation is usually with anaemia, leucopenia, thrombocytopenia and massive splenomegaly. Overlap with other myeloproliferative diseases may also produce high blood cell counts. Bone marrow fibroblasts are nonclonal and bone marrow fibrosis is most likely the consequence of increased production of mitogenic growth factors such as platelet-derived growth factor and transforming growth factor β by the malignant clone (platelets/ monocyte/ macrophages). Early stage disease involves intramedullary clonal haematopoietic expansion ('cellular phase') followed by increasing bone marrow fibrosis and extramedullary haematopoiesis. Extramedullary haematopoiesis is found predominantly in the spleen and liver, but adrenal, kidney, endometrial and pulmonary sites are also reported. Stem cell migration from bone marrow to spleen is more likely than reactivation of spleen-derived stem cells. Reduced splenic filtration and phagocytic capacity suggested by reduced CD68+ splenic cordal macrophages, may allow the establishment of splenic haematopoiesis. Haematopoiesis is ineffective and associated with increased splenic pro-erythoblasts Ki-67 and PCNA expression, suggestive of S-phase prolongation as in other bone marrow disorders where ineffective haematopoiesis predominates. Myeloproliferative splenic haematopoiesis favours the granulocytic lineage. Circulating pluripotent (CFU-GEMM) and committed progenitors (CFU-GM,BFU-E, CFU-Mk) are paradoxically increased in number, with normalisation postsplenectomy. Autonomous progenitor growth (CFU-Mk, BFU-E) is identified in up to 80% of patients. In conclusion, despite the establishment of extramedullary haematopoiesis in agnogenic myeloid metaplasia, the clonal abnormality renders blood cell production ineffective and is insufficient to sustain normal adult values of circulating mature blood cells.

POSTERS

D1 Extinction of myeloid metaplasia in the immediate vicinity of a benign haemangioma in the liver of a patient with long-lasting primary myelofibrosis/sclerosis. By K. Jakab, M. Fanossa and E. Kelemen (introduced by D. B. Thomas). *National Institute of Haematology, Budapest, Hungary*

This report is based on the case of a 48 y old patient in the 1st Dept of Medicine, Semmelweis University Medical School, Budapest, who had been suffering from primary myelofibrosis for 28 y. He was splenectomised in 1989 and died in 1991. Morphological studies confirmed the clinical diagnosis. Remarkable osteosclerosis developed during the long disease process, and the liver was the site of overwhelming diffuse trilinear myeloid metaplasia. As a chance observation, a single, walnut-sized benign haemangioma was found in the enlarged liver. Histological studies showed that unlike all other zones, practically every cell of the intercolumnar, trilinear myelopoiesis disappeared from the immediate vicinity of the tumour, within a radius of 300–2000 μm. Only red cells were detectable in these areas. The reasons for this finding are unclear but mechanical factors, local hypoxia, or chemical alterations may be important.

D2 Expression of the sialomucin CD34 during early mouse cardiovascular development and haematopoiesis. By H. B. Wood, *G. May, *L. Healy, *T. Enver and G. M. Morriss-Kay. *Department of Human Anatomy, University of Oxford and the Leukaemia Research Fund Centre at The Institute of Cancer Research, Chester Beatty Laboratories, London*

CD34 is a 105–120 kDa cell surface transmembrane glycoprotein that is selectively expressed within the human haematopoietic system on stem and progenitor cells; expression is lost as the cells mature. It is thought to have a role in adhesion. We have analysed the expression pattern of CD34 during early mouse embryonic development at the RNA level using in situ hybridisation and at the protein level using a recently developed monclonal antibody (RAM 34), which we show reacts with murine CD34 epitopes in a glycosylation-independent manner. CD34 RNA transcripts and (at slightly later stages) protein were observed in pre-endothelial cells of the yolk sac and embryo, suggesting a possible role in the formation of blood vessels de novo (vasculogenesis). Expression was lost from vessels forming by coalescence, including the yolk sac vessels and the cardiac veins. Strong staining for both RNA transcripts and protein was observed in vessels forming by capillary sprouting (angiogenesis), except for the second and subsequent aortic arches. Only a minority of the erythropoietic cells of the yolk sac blood islands was positive for CD34 protein. As the yolk sac circulation began to form, CD34-

https://doi.org/10.1046/j.1469-7580.1997.19020311.x Published online by Cambridge University Press

positive haematopoietic cells were visible within the vitelline vessels. Their numbers declined between d 9 and 10 of development, the period during which haematopoiesis switches from the yolk sac to the embryo. On d 10, CD34-positive cells were observed in the mesenchyme of the liver. Scattered CD34-positive cells were also seen in the para-aortic mesenchyme, and attached to the very strongly CD34-positive endothelial lining of the dorsal aortae; these are thought to be the earliest sites of intra-embryonic haematopoiesis. These results support evidence from previous studies for separate origins of haematopoietic and endothelial cell lineages, except that the endothelium of the dorsal aortae may have a haemangioblastic function that is not localised to a specific site.

https://doi.org/10.1046/j.1469-7580.1997.19020311.x Published online by Cambridge University Press