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Hematopoietic mutations in the zebrafish

Brant M. Weinstein, Alexander F. Schier, Salim Abdelilah, Jarema Malicki, Lilliana Solnica-Krezel, Derek L. Stemple, Didier Y. R. Stainier†, Fried Zwartkruis‡, Wolfgang Driever and Mark C. Fishman*

Cardiovascular Research Center, Massachusetts General Hospital, 149 13th Street, Charlestown, MA 02129, USA and Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

†Present address: Department of Biochemistry and Biophysics and Program in Developmental Biology, University of California, San Francisco, San Francisco, CA, 94143-0554, USA
‡Present address: Laboratory for Physiological Chemistry, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands
*Author for correspondence

SUMMARY

We have identified mutations that perturb the formation or differentiation of the first embryonic blood cells in the zebrafish embryo. These 'primitive' red blood cells originate in the intermediate cell mass of the trunk, a derivative of the dorsal lateral plate mesoderm. By transfusion of blood between embryos we demonstrate that this cohort of cells provides the embryo with all, or nearly all, of its blood cells until at least day 5 postfertilization. Larval lethal mutations generated by ENU mutagenesis affect different steps in the development of these cells. Some cause defects in precursor generation, others defects in differentiation, and others an increase in cellular photosensitivity.

Key words: primitive haematopoiesis, mutations, zebrafish

INTRODUCTION

Blood cells are generated in sequentially different anatomical sites during embryonic development. Although relatively well-understood in the adult, or 'definitive', locale, such as the bone marrow in humans, the molecular nature of the process is less well defined with regard to the developmentally earlier or 'primitive' sites. In many vertebrates the first mature blood cells, all erythrocytes, originate in the blood islands of the yolk. Soon thereafter blood cell formation begins within the embryo proper; in many vertebrates this 'intraembryonic' site is believed to be in the mesoderm near to the aorta (Dieterlen-Lievre and Martin, 1981; Godin et al., 1993; Medvinsky et al., 1993; Turpen et al., 1981). These early populations are of interest because of their potential harboring of pluripotential stem cells. In most vertebrates, screening for genetic defects affecting early blood cell lineages is difficult because their embryos do not survive without blood.

Zebrafish embryos are externally fertilized and optically transparent, and blood cell formation and differentiation can be assessed throughout early development. This makes the zebrafish ideal for genetic and experimental embryologic analysis of early stages of hematopoiesis. The blood cells of fish in general and of the taxonomic superorder Teleosti (including catfish, cyprinids such as carp and zebrafish, flatfish, eels, salmonids, and blennies), in particular, resemble those of other vertebrates (for a review of fish blood cells, see Rowley et al., 1988). Teleosts possess erythrocytes, monocytes, granulocytes, and thrombocytes. Their hemoglobin has the typical higher vertebrate $\alpha_2\beta_2$ quaternary structure. The adult erythocytes of fish, birds, amphibians and reptiles are nucleated, unlike those of mammals (Gulliver, 1875), although the first or 'primitive' embryonic blood cells in mammals are nucleated also (Cohen et al., 1990). Secreted complement factors, interferons, leukotrienes, and prostaglandins appear to be present in fish (Rowley et al., 1988), and homologs of intracellular hematopoietic regulatory molecules such as the GATA factors have been identified (Detrich et al., 1995).

Here we examine the development of the earliest hematopoietic cells in zebrafish embryos and describe mutations which disrupt their development.

MATERIALS AND METHODS

Zebrafish methods and isolation of mutants

Zebrafish were raised, maintained and staged as described by Kimmel et al. (1995) and Westerfield (1994). The methods employed to generate and recover the mutants described in this paper are given in detail by Driever et al. (1996). Mutations were identified by visual screening. Genetic complementation was tested by pairwise crosses between heterozygotes. The progeny of at least two separate crosses were examined. All complementation groups assigned locus names were tested against one another. Mutations for which complementation analysis is incomplete have not been assigned a locus name, and are designated with (–).

Microscopy and histology

Photomicroscopy was performed on an Axiophot (Zeiss) microscope equipped with DIC optics, or on an M10 (Wild) dissecting microscope. Videomicroscopy was performed on an Axioskop (Zeiss) equipped with DIC optics and a SIT camera (Dage-MTI). Images of photosensitive blood cells in dark-raised mutants were taken using extremely low transmitted light illumination to prevent blood cell lysis during photography, averaging 128 frames or more using Metamorph imaging software (Universal Imaging).

Dechorionated embryos were fixed for histology, overnight at 4°C using BT fixative (Westerfield, 1994), embedded in JB4 (Polysciences), and sectioned at 5 μm. JB4 sections were stained with Wright-Giemsa (Sigma) or methylene blue (Polysciences). Diaminofluorene (DAF) staining was performed to test for the activity of hemoglobin peroxidase (modified from Miwa et al., 1991). Embryos were fixed for 30 minutes at room temperature in BT fixative (Westerfield, 1994), washed 3 times with PBS, and preincubated in DAF staining solution (0.01% diaminofluorene, 200 mM Tris, pH 7.0, 0.05% Tween-20) for 30 minutes to 1 hour at room temperature in the dark. 30% hydrogen peroxide was added to a final concentration of 0.3%, and the embryos were incubated for a further 5-20 minutes. Embryos were washed 3 times in PBS, fixed further in BT fixative overnight at 4°C, and then washed three times in PBS. DAF-stained embryos were photographed in whole mount and/or embedded in JB4 for sectioning.

Embryonic blood transfusions

Embryos were obtained from crosses between adult wild-type zebrafish. Donor embryos were injected at the 1- to 8-cell stages with 1% rhodamine-dextran (Molecular Probes) in 200 mM KCl. Transfusions of blood cells from donor to host embryos were performed at 1.25 to 1.5 days postfertilization (dpf), after the initiation of circulation. Transfusion microneedles were prepared by pulling fine needles from 1.0 mm OD borosilicate glass capillaries without a filament (World Precision Instruments) on a vertical pipette puller and then breaking off the tips at a inner diameter of approximately 10 μm. Needles were secured to a pipette holder attached to a pressure control station (Eppendorf); withdrawal and expulsion of cells was controlled by increasing or decreasing air pressure to the needle. Donor and host embryos were anesthetized with tricaine (Sigma, method in Westerfield, 1994), and immobilized with a holding pipette applied to the side of the yolk ball in a depression-well slide filled with embryo medium (Westerfield, 1994) supplemented with tricaine and approximately 20 units/ml heparin (Sigma). A small amount of this solution was usually present in the tip of the needle before insertion into the donor embryo just posterior to the sinus venosus. From 1% to 50% or more of the blood cells in donor embryos were removed and transfused into a single host embryo. Both donor and host embryos survived the transfusion apparently unharmed.

On the day after transfusion and subsequent days, the fraction of circulating cells containing fluorescent dye was scored in host embryos by temporarily stopping the heart with a tricaine overdose to halt circulation and then counting the number of labelled and unlabelled cells in caudal portions of the axial artery. Embryos were quickly returned to medium without tricaine after counting and all recovered their heartbeat and circulation within a minute or two. Eight different transfused hosts were used to generate the results shown in Fig. 2. The fraction labelled at each time point for each of the eight embryos is normalized to the day 2 fraction for that embryo, and the normalized values for each time point are then averaged.

RESULTS

Generation of embryonic blood cells in normal zebrafish embryos

The first cohort of blood cells in the embryonic zebrafish develops entirely within the embryo proper, in contrast to yolk sac hematopoiesis in mammals, birds, and many other fish. Blood cells form in the trunk ventral to the notochord in conjunction with the major axial vessels in the intermediate cell mass (IMC) (Al-adhami and Kunz, 1977). As in other vertebrates (Cohen et al., 1990), erythrocytes appear to be the only type of mature blood cells generated in this first, or primitive, wave of hematopoiesis. By the 22 somite stage (approximately 20 hpf; hours postfertilization) presumptive proerythroblast-like cells, approximately 9 μm in diameter with a large nucleus, are seen in histological sections through the IMC (Fig. 1A). Heme synthesis is initiated by the 25 somite stage (21.5 hpf), as indicated by weak staining for hemoglobin peroxidase activity with diaminofluorene (DAF). By the 28 somite stage (23 hpf), DAF staining is easily detectable throughout the IMC (Fig. 3C). Immature blood cells migrate onto the yolk sac at about 24 hours and begin to circulate shortly thereafter. Blood cells are still in the large erythroblast stage when circulation begins (Fig. 1B). Over the next 4 days the blood cells assume a mature elliptical form (Fig. 1C,D,E), although they are clearly distinguishable from adult zebrafish erythrocytes that have a characteristic large elongated nucleus and smaller amount of cytoplasm (Fig. 1F).

This first cohort of IMC-derived blood cells persists as the only circulating cells for at least 4 days. We demonstrated this by transfusion of fluorescently labelled 1.5-day old wild-type donor embryo cells into an unlabelled 1.5-day old wild-type host embryo (Fig. 2B). The proportion of blood cells in the circulation which are contributed by the donor does not decline significantly until approximately 6 dpf. After 5 dpf, both the proportion and number of donor blood cells decline gradually, reaching approximately 50% of their starting values by 10 dpf (Fig. 2A). At 10 days, labelled transfused erythrocytes are noticeably smaller in size than many unlabelled erythrocytes, indicating the emergence of a morphologically distinctive new erythrocyte cell population (data not shown). These results indicate that a single population of blood cells, originating in the IMC, forms and matures during the first 5 days of development in zebrafish embryos.

Mutations affecting embryonic blood cells in zebrafish

Mutations affecting blood cell development were isolated as part of a large-scale screen for mutations affecting embryonic development in the zebrafish (Driever et al., 1996). The mutant phenotypes are summarized in Table 1 with the results of pairwise complementation analysis, and described in more detail below. The mutations are grouped into three categories on the basis of their cellular phenotype. The first group includes mutants defective in blood cell generation, including *vampire (vmp)*, *vlad tepes (vlt)*, *m547* and *m594*. The second group includes mutants defective in blood cell differentiation and/or maintenance, including *sticky blood (sti)*, *pale and wan (paw)* and *clear blood (clb)*. Blood cells are grossly normal in both number and morphology at 1-2 dpf, but become abnormal thereafter. The third group includes mutants with photosensitive blood cells, including *dracula (drc)* and *desmodius (dsm)*. When raised in normal room light, homozygotes lack virtually any circulating cells, but when they are raised in the dark nearly normal numbers of blood cells develop.

Mutations affecting blood cell generation

This group of mutants generate no, or very few blood cells at any time during the first 5 days of development. They appear to develop fairly normally for at least the first 5 days, despite

Table 1. Zebrafish blood mutants

Genetic loci	Alleles	Phenotype	Other phenotypes
Group I. Generation mutants			
vampire (vmp)	*m62, m262*	Absent or reduced blood cells from 1d on	
vlad tepes (vlt)	*m651*	As for *vmp*	
(–)	*m547*	Reduced blood cell number from 1d on, at least partially dominant	
(–)	*m594*	Reduced blood cell number from 1d on	
Group II. Differentiation/maintenance mutants			
sticky blood (sti)	*m232*	Blood cells fail to mature, number strongly reduced after 2d	
pale and wan (paw)	*m416*	Blood cells fail to mature, number reduced after 3d	
clear blood (clb)	*m525*	Hypochromic blood cells after 2d	
Group III. Photosensitivity mutants			
dracula (drc)	*m87, m159, m248, m328*	Virtually no blood cells in light, photosensitive blood cells in dark	
desmodius (dsm)	*m534*	As for *drc*, but a few aberrant cells present in light	Edema and heart defects in most

the blood cell defect. Blood vessels form normally. However, homozygous larvae fail to inflate their swim bladders and die within the first 2 weeks.

The mutations are recessive, with the exception of *m547*. Expressivity and/or penetrance of mutations in this group appears to be dependent on the genetic background. vmp^{m62}, for example, segregates as a fully penetrant, fully expressive mutation through several generations of outcrossing into the AB background (i.e., 25% of embryos from heterozygous matings have less than 1% the normal blood cell number. The rest of the embryos appear normal). When crossed into a hybrid AB/India background, however, the mutation shows reduced penetrance and expressivity in many crosses (for example, in 6 crosses between different hybrid siblings heterozygous for vmp^{m62}, the percentage of embryos scored as mutants varied between 12% and 28%, and many of these embryos had greater than 1% of normal blood cell numbers). The *m547* mutation is at least partially dominant, with variable expressivity. In an outcross of one heterozygote to wild type, 9% of the embryos had reduced blood cell numbers. In crosses between two heterozygotes, 46% of embryos were scored as mutant. In either case, affected embryos had variable numbers of blood cells, from no blood cells to nearly normal levels.

Prior to the commencement of circulation, vmp^{m62} and vlt^{m651} homozygotes are marked by severe reduction in blood cell precursors and accumulation of debris in the IMC (Fig. 3B). Heme synthesis in the IMC, as assessed in an unaffected embryo by staining with DAF (Fig. 3C), is not detected in a mutant sibling (Fig. 3D). After the commencement of circulation, the rare circulating cells that are found in the bloodstream neither assume the morphology of mature erythrocytes nor acquire DAF staining (Fig. 3F).

Mutations affecting blood cell differentiation and maintenance

This group of mutants generates blood cells that are grossly normal in number and appearance at 1 dpf but that show abnormalities by 2 dpf. They are all recessive lethal and fully penetrant. Vascular development appears normal.

In sti^{m232} mutants (Fig. 4B) the first noticeable abnormality is the failure of the blood cells to mature from a round to lentiform shape, which normally begins to occur at about 1.5-2 dpf (Fig. 4A). Thereafter, blood cells are rapidly lost from the circulation, so that they are greatly reduced by 3 days and almost entirely absent by four. The cells circulating at 2-4 dpf remain strongly DAF positive. Between 2 and 4 dpf cells can be seen apparently adhering to the endothelium of blood vessels, particularly in the caudal vein (data not shown).

Like sti^{m232} mutants, blood cells in paw^{m416} homozygotes do not mature morphologically (Fig. 4C) and heme synthesis appears to be maintained (data not shown). Blood cells in paw^{m416} persist in the circulation for a longer time than do those of sti^{m232}; the number of circulating blood cells at day 3 is 10-25% and at day 7 is 1-5% of normal.

The phenotype observed in clb^{m525} homozygotes differs from that of the other two mutations in this category. Morphological maturation into lentiform erythrocytes does occur, at least partially, but the cells are abnormal in shape (Fig. 5B). At 1.25 dpf, heme synthesis appears to be normal (data not shown) but it is strongly reduced at later stages. By 3 dpf, when circulating cells are visibly red in wild-type embryos, blood cells in the mutants remain colorless, although weakly DAF positive (Fig. 5C).

Mutations causing blood cell photosensitivity

The hallmark of this group of mutations is the presence of highly photosensitive circulating cells. Raised normally in the light, most of the *drc* mutants have no or virtually no circulating cells (Fig. 6B). drc^{m159} embryos initially lack blood cells, but begin to acquire them at about 4 dpf, although at markedly diminished levels. Apart from the blood cell phenotype, *drc* mutants are otherwise grossly fairly normal over the first 5 days of development. dsm^{m534} mutants have slightly more blood cells than do *drc* mutants and also display more non-hematopoietic abnormalities, including severe pericardial edema and cardiac dysfunction. Blood vessels in both drc^{m87} and dsm^{m534} mutants appear normal.

Raised in total darkness, both drc^{m87} and dsm^{m534} embryos have nearly normal numbers of blood cells (Fig. 6C,D). Blood cells in dark-raised homozygous mutants are acutely photosensitive. Upon exposure to strong illumination, blood cells in dracula homozygotes lyse within seconds, and surrounding tissue frequently becomes necrotic (data not shown). Blood cells in dsm^{m534} homozygotes are somewhat less photosensitive than those in drc^{m87} embryos, requiring several minutes for complete lysis. Even when they are maintained in total darkness, homozygous embryos of both groups eventually lose their blood cells and do not survive past larval stages.

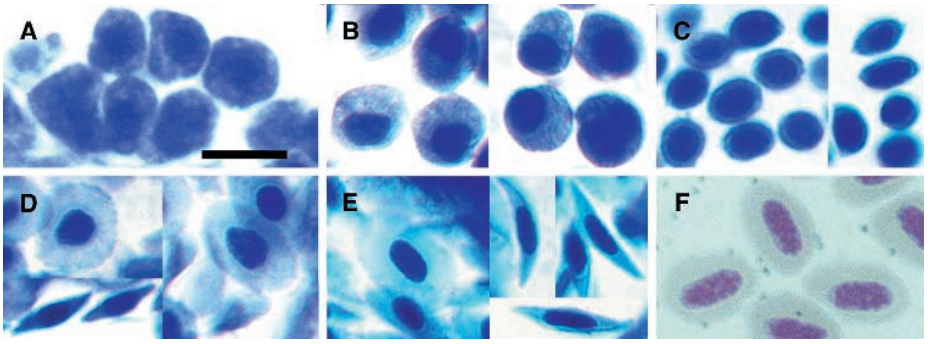

Fig. 1. Blood cell differentiation in wild-type zebrafish embryos during the first 5 days of development. (A-E) Wright-Giemsa (A) or methylene blue (B-E) stained longitudinal sections through the trunk of embryos at various stages. Embryos were obtained from a cross between two wild-type adult zebrafish. (A) 23 somite (20.5 hpf) embryo; (B) 24 hour embryo; (C) 36 hour embryo; (D) 2.25 dpf embryo; (E) 5 dpf embryo; (F) Wright-Giemsa stained blood smear from an adult fish. Scale bar, 10 μm.

DISCUSSION

The first blood cells in zebrafish embryos develop in the IMC

The first circulating cells in the zebrafish are erythrocytes arising in a single synchronous wave of development. These first or so-called primitive erythrocytes originate in the intermediate cell mass (IMC) of the trunk together with the systemic trunk vasculature, rather than in blood islands on the yolk sac, as in most other vertebrates. Presumptive proerythroblast-like cells are seen in this region by the 22 somite (20 hpf) stage, and heme synthesis thoughout this region is detectable by 25 somites (21.5 hpf) using DAF staining. Immature erythroblasts begin to circulate at 1 dpf and mature into their final nucleated form over the next several days.

The IMC origin of the first primitive hematopoietic series in zebrafish contrasts with the yolk-sac origin of these cells in most other vertebrates, and may resemble more the paraxial mesodermal origin demonstrated for the first intraembryonic

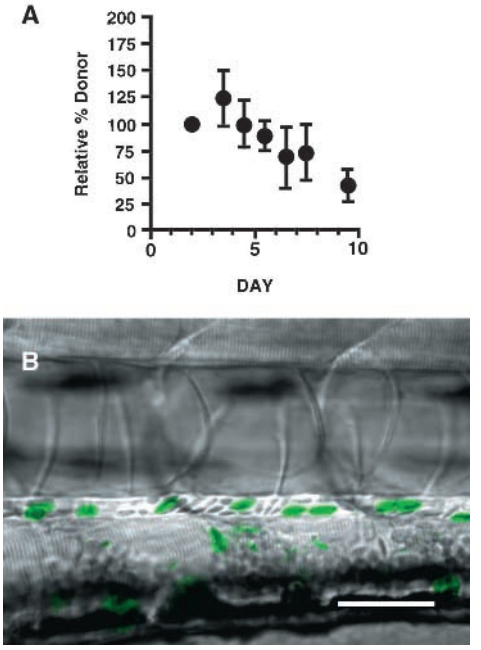

Fig. 2. Persistence of donor blood cells in host embryos transfused at 1.5 dpf. Blood cells were removed from fluorescently labelled wild-type donor embryos and transfused into 8 different unlabelled wild-type host embryos. The fraction of the cells in circulation contributed by the donor embryo (i. e., fluorescently labelled) was tabulated at intervals thereafter. Values were normalized to the starting fractions measured at 2 days, and then averaged together for each time point. (A) Normalized, averaged values are shown as percentages of the starting value at 2 dpf (100%). Error bars show the standard error of the data. (B) Blood cells in the caudal artery of a 5 dpf transfused host. A fluorescence videomicrograph was psuedocolored green and overlaid on the corresponding DIC image of the same field. Labelled blood cells are normally developed for their age. Scale bar, 50 μm.

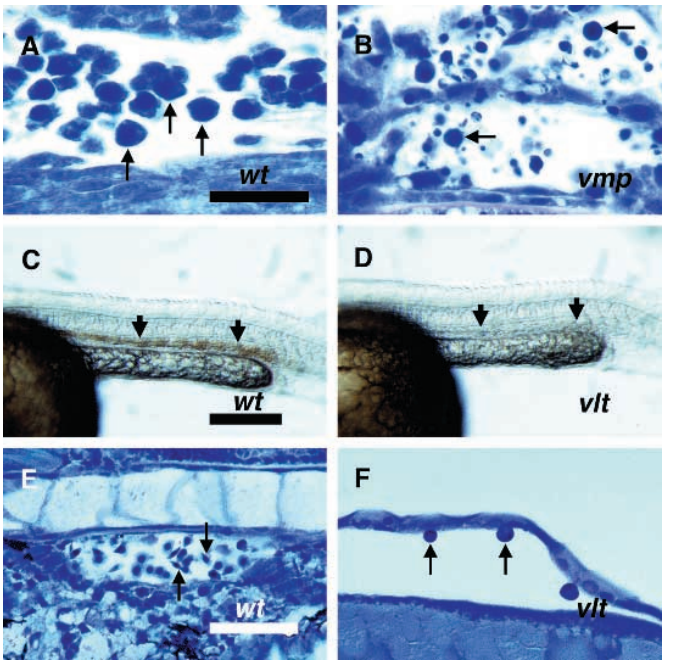

Fig. 3. Blood cells are not properly generated during early stages of hematopoiesis in *vmp* and *vlt* mutants. Embryos were obtained from crosses between two vmp^{m62} heterozygotes (A,B) or two vlt^{m651} heterozygotes (C-F). (A,C,E) Phenotypically wild-type embryos. (B,D,F) Mutant siblings. (A,B) Wright-Giemsa stained longitudinal sections from the tails of 27 somite stage (approximately 22.5 hpf) mutant (B) and unaffected sibling (A) embryos. Erythroblasts are seen in both embryos (arrows), but there are many fewer are present in mutants. (C,D) Whole DAF stained 28 somite stage (23 hpf) phenotypically wild-type (C) and mutant (D) embryos. DAF staining is seen in the IMC of wild-type but not mutant embryos (arrows). (E,F) Wright-Giemsa stained longitudinal sections of 2.25 dpf phenotypically wild-type (E) and mutant (F) embryos. Normal blood cells are seen in wild-type embryos, but only abnormal, large cells are seen rarely in mutants (arrows). Scale bars 25 μm (A,B), 200 μm (C,D) and 50 μm (E,F).

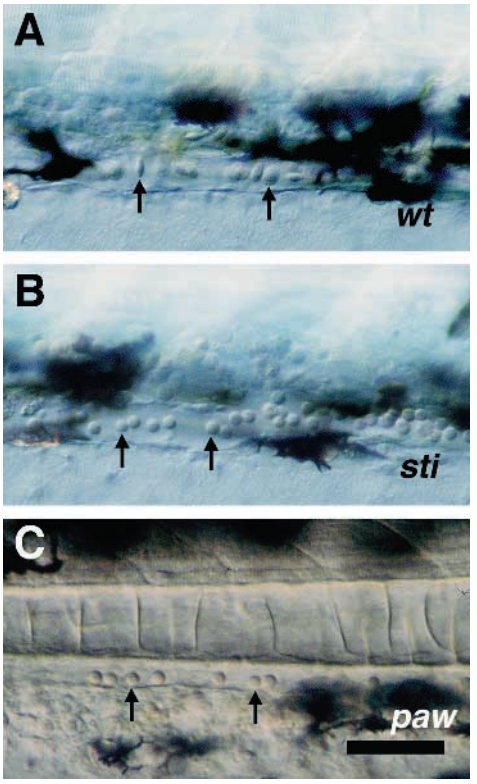

Fig. 4. Defects in erythrocyte morphogenesis in *sti* and *paw* mutants. DIC photomicroscopy of blood cells (arrows) in tail vessels of zebrafish embryos. (A) Phenotypically wild-type 2.25 dpf embryo from a cross between two *sti*^{m232} heterozygotes; (B) mutant 2.25 dpf embryo from a cross between two *sti*^{m232} heterozygotes; (C) mutant 2.25 dpf embryo from a cross between two *paw*^{m416} heterozygotes. Scale bar, 50 μm.

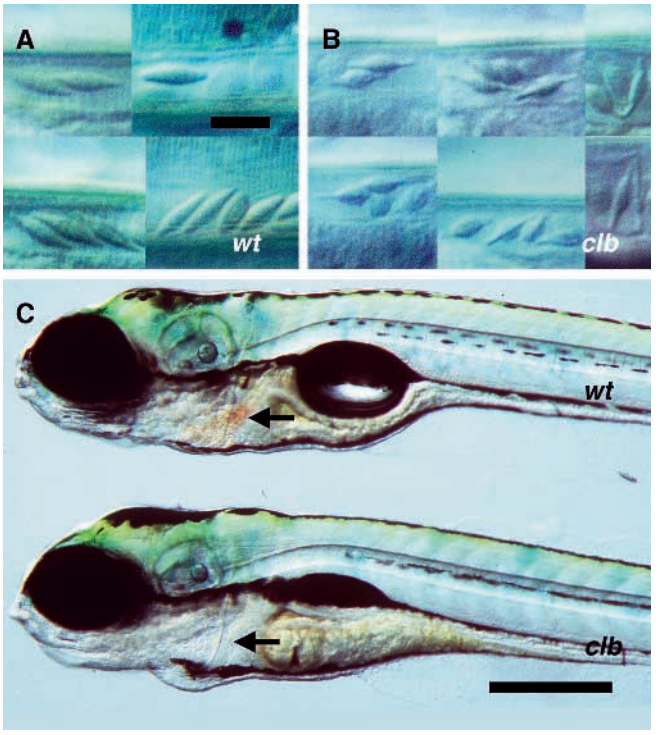

Fig. 5. Defects in erythrocyte morphology and hemoglobin accumulation in *clb* mutants. Embryos were obtained from a cross between two *clb*^{m525} heterozygotes. (A,B) DIC photomicroscopy of blood cells in the caudal arteries of 5 dpf phenotypically wild-type (A) and mutant (B) embryos. (C) Photomicrograph of anterior portions of live 3-day old phenotypically wild-type (top) and mutant (bottom) embryos. The heart appears visibly red (arrow) in the wild-type but not the mutant embryo (arrows). Scale bars, 10 μm (A,B), 400 μm (C).

hematopoietic lineages in amphibians (Turpen et al., 1981), birds (Dieterlen-Lievre and Martin, 1981), and mammals (Godin et al., 1993; Medvinsky et al., 1993). In the zebrafish, these IMC-derived cells constitute the first wave of erythropoietic development, and the only detectable one for about the first 4 days, as we demonstrate by embryonic blood cell transfusion. Transfusion experiments and histology indicate that a new, larger and more adult-appearing erythrocyte population comes to predominate in the circulation by 10 dpf. The source of these cells is not known, although a previous report suggests that they may be derived from the endocardium of the heart (Al-adhami and Kunz, 1977). By adulthood, it is believed that the definitive source of erythrocytes in the zebrafish is the kidney (Al-adhami and Kunz, 1977). All of the blood mutants that we have isolated affect the IMC-derived lineage. Because of the early embryonic lethality of these mutants we cannot at present comment on their effects on later-initiating blood cell sources.

Mutations affecting blood cell generation

In two of these mutants, *vmp* and *vlt*, hematopoietic progenitors to the primitive lineage do not form at all or are rapidly destroyed at or prior to formation of proerythroblasts in the IMC (as assessed by histology and by the absence of DAF staining). One possibility is that these mutations disrupt known crucial regulators of early hematopoietic development. Candidates for such regulators include growth factors and receptors known to support hematopoietic lineages, or transcription factors necessary for their differentiation. Although most of these have been defined by their effects upon cultured cell lines or blood from adult hematopoietic sites, the developmentally early effects of mutants and targeted gene ablations in mice suggest that some may affect embryonic lineages as well. For example, anemia is present in embryos homozygous for mutations at the *steel* and *white* loci, which encode respectively a trophic factor secreted by marrow stroma necessary for the survival of pluripotent hematopoietic stem cells and a receptor (c-kit) for this factor (Blume-Jensen et al., 1995). Deficiencies of primitive red blood cells are noted at embryonic day 9 in mice with targeted mutations of the genes for the transcription factors GATA-1 (Pevny et al., 1991; Weiss et al., 1994), GATA-2 (Tsai et al., 1994), SCL/Tal-1 (Shivdasani et al., 1995), and Ttg-2/rbtn-2 (Warren et al., 1994). Other mutations which may act even earlier during development affect both haematopoietic and endothelial development. Targeted mutation in mice of a VEGF receptor, *flk-1*, prevents formation of both blood cells and endothelial cells, either on the yolk or in the embryo proper (Shalaby et al., 1995). In the zebrafish mutation, *cloche*, both blood and vascular lineages are markedly reduced (Stainier et al., 1995). These genes might independently affect the hematopoietic and endothelial lineages, or they could act upon a shared precursor cell, the

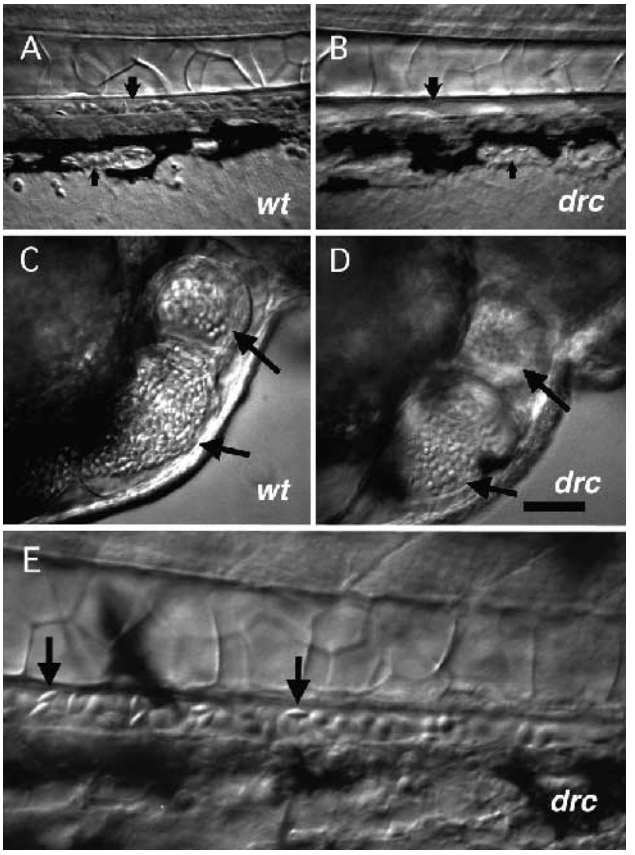

Fig. 6. Blood cells are photosensitive in *drc* mutants. (A-D) DIC videomicroscopy of 2.5 dpf embryos using low-light level illumination and a SIT camera with frame-averaging. All embryos were obtained from a single cross between two *drc*^{m87} heterozygotes. Circulation was stopped in the embryos by tricaine overdose. (A) The tail of a phenotypically wild-type embryo raised continuously in room light. Blood cells are seen in the caudal artery (large arrow) and caudal vein (small arrow). (B) The tail of a mutant sibling raised continuously in room light The artery and vein are both present (arrows), but contain no blood cells. (C) Blood cells in the heart of a phenotypically wild-type embryo raised in darkness from blastula stages on. (D) Blood cells in the heart of a mutant sibling embryo raised in darkness from blastula stages on. Abundant blood cells are seen in both the phenotypically wild-type and mutant embryos (arrows). (E) Higher magnification view of blood cells in the caudal artery of a mutant embryo. Blood cells with the typical lentiform morphology for this age are seen (arrows). Scale bar, 100 μm (A-D), 29 μm (E).

'hemangioblast' (Wagner, 1980). If the latter is the case then the mutations reported here, all of which retain an intact vasculature, probably act downstream of *cloche*.

Embryonic survival in mammals depends upon blood, so it is unlikely that mutations that disrupt primitive blood cell generation would be found in humans, at least not in fully expressive form. Aplastic anemia does occur rarely as a congenital disorder in humans (Saunders and Freedman, 1978), but usually manifests itself only later during childhood, and is generally caused by a markedly enhanced sensitivity of cells to DNA damage (Bloom et al., 1966; German et al., 1987; Rosendorff and Bernstein, 1988). Acquired aplastic anemias and hypoplastic anemias associated with other diseases are much more common, however, and mutations in factors regulating early hematopoietic stem cells would be of great interest in helping to understand how the maintenance and replenishment of these cells is perturbed by disease.

Mutations affecting blood cell differentiation

The arrest of blood cell development in *sti*^{m232} and *paw*^{m416} is manifested by failure to mature from round to lentiform shape, and accompanied by rapid decrement in blood cell number after day 2. It is likely that the rapid loss of these cells is due to abnormally accelerated hemolysis, since blood cell transfusion demonstrates that the first wave of blood cells is normally much longer-lived and additional sources do not become quantitatively evident until after day five. The presence of apparently immobile blood cells in the vessels of *sti*^{m232} homozygotes suggests that the defect is accompanied by abnormal adherence to the endothelium. In humans, red cell membrane defects are one cause of enhanced hemolysis (Williams et al., 1990). The hypochromic and anisotropic morphology of red blood cells in *clb*^{m525} mutants is similar to that of thalassemias (Weatherall, 1990) or defects in iron metabolism such as atransferrinemia (Bernstein, 1987; Goya et al., 1972).

Mutations causing light-induced hemolysis

Homozygous *drc* ^{m87} and *dsm*^{m534} mutants have relatively normal levels of blood cells if raised in total darkness. Illumination of dark-raised mutants causes acute hemolysis. These embryos may contain light-sensitive pigments due to mutations in the heme biosynthetic pathway. Deficiencies of some of the heme biosynthetic enzymes in other species result in accumulation of metabolic intermediates, or their oxidized forms, known collectively as porphyrins, causing diseases termed porphyrias (reviewed by Meyer, 1991). Porphyrins absorb light in the 400 nm range and release oxidative by-products that damage the cell.

Conclusion

Although much is known about hematopoiesis, the mechanism of differentiation of the early population of primitive blood cells may differ from that of the far better-studied definitive lineages. It is precisely because so little is known about the formation of these earliest populations of blood cells that we hope these mutations will provide insight into the earliest decisions of hematopoiesis.

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