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Control of Hematopoietic Cell Growth Regulators during Mouse Fetal Development

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Gene expression for the four different growth-regulatory proteins for cells of the myeloid hematopoietic cell lineages was analyzed in mouse fetal and extraembryonic tissues at various stages of development. The macrophage growth inducer MGI-1M (colony-stimulating factor 1) was the only myeloid hematopoietic growth regulator detected as both mRNA and bioactive protein during fetal development. This regulator was produced predominantly in extraembryonic tissues, and the production of hematopoietic growth regulators in embryogenesis was regulated by transcriptional and posttranscriptional controls.

Normal precursor cells of the myeloid hematopoietic lineages require specific growth-inducing proteins for cell viability and proliferation. Four different growth inducers for myeloid cells have been identified. These are called macrophage and granulocyte inducers type 1 (MGI-1) or colony-stimulating factors (CSFs), and one is also called interleukin 3 (IL-3) (reviewed in references 16, 22, 23, 23a, and 24). Of these four growth-inducing proteins, one (MGI-1M, also called macrophage CSF and CSF-1) induces the development of normal cell clones with macrophages, a second (MGI-1G, also called granulocyte CSF) clones with granulocytes, a third (MGI-1GM, also called granulocyte-macrophage CSF) clones with both macrophages and granulocytes, and a fourth (IL-3) clones with macrophages, granulocytes, mast cells, eosinophils, erythroid cells, and mega-karyocytes. The cDNA coding for the four myeloid growth-inducing proteins (murine and human) has been cloned, and each of these four proteins is coded for by a different gene (7, 8, 11, 12, 17, 27, 30–32). The availability of these cDNA clones and assays for the different proteins have enabled us to examine the developmental expression of these growth inducers in terms of mRNA as well as protein.

Expression of mRNA. From C3H/eBJ mice at different gestational ages, mRNAs were extracted (1) from placenta (days 8 to 18), fetal membranes, whole fetuses (days 10 to 18), and fetal organs (days 14 and 18). Samples of polyadenylated [poly $(A)^+$] RNA obtained by separation on oligo(dT)-cellulose (2) were run on denaturing agarose gels (13) and transferred onto nitrocellulose paper (18). The filters were hybridized (28) with four different nick-translated (21) cDNA probes coding for the specific myeloid cell growth factors IL-3 (32), MGI-1GM (8), MGI-1M (11), and MGI-1G (17). A summary of the results obtained with RNA samples during fetal development is presented in Table 1. The only mature mRNAs species detected by our screening were the mRNAs reactive with the specific cDNA probe for MGI-1M. The human MGI-1M cDNA probe used hybridized with several transcripts ranging in size from about 1.5 to 4.5 kilobases (kb) (11). The specific pattern we obtained varied among tissues but was consistent for different samples derived from the same tissue types. Thus, placental mRNAs showed three specific bands, whereas fetal membranes showed only two specific bands. Poly$(A)^+$ RNA from fetal membranes and placenta gave specific hybridization with this MGI-1M-specific probe at all gestation ages tested, (Fig. 1). mRNA samples from whole fetuses or from dissected fetal lungs or liver revealed low levels of MGI-1M-specific transcripts.

The human MGI-1G cDNA probe hybridized with an mRNA of approximately 1.6 kb in human and mouse tumor cell lines (17, 27). The mRNAs from whole or partially dissected fetuses and from placentas did not hybridize to this MGI-1G probe. However, strong hybridization to a band of approximately 2.8 kb was obtained with poly$(A)^+$ RNA samples extracted from yolk sac and amnion from 10 days of gestation until birth (Fig. 2). This 2.8-kb transcript may be a precursor of the mature mRNA species.

Poly$(A)^+$ RNA samples from whole or partially dissected fetuses, yolk sac and amnion, and placenta from different times of gestation until birth failed to reveal any detectable specific transcripts of mouse IL-3 (Fig. 3A) or mouse MGI-1GM (Fig. 3B). To increase the sensitivity of detection, these two cloned cDNAs were also used as templates to produce single-stranded RNA probes by using the SP6 promoter system (15). The results were the same with this sensitive detection system. As positive controls for hybridization, WEHI-3 cells were used as a source of IL-3 (5, 9) and lungs from endotoxin-treated mice were used as a source of MGI-1GM (4). The quality of the poly$(A)^+$ RNA from the fetal tissues was also tested by hybridization to a cloned probe specific for β-actin (19). This probe hybridized with a single specific mRNA of 2kb found in virtually constant amounts in samples of poly$(A)^+$ RNA from mice of all gestational ages, as expected.

Production of growth inducer protein. The amniotic fluid and extracts of fetal and extraembryonic tissues from C3H/eBJ mice were assayed (20) for their ability to induce cluster and colony formation in cultures of mouse bone marrow cells. Of all the extracts (6) and fluids tested, the highest levels of MGI-1 activity were found in the amniotic fluid. The level of MGI-1 was already high at 9 days; maximum activity was obtained at 11 to 12 days of gestation and decreased gradually until birth (Fig. 4). The next-richest source of protein activity was the extract of yolk sac and amnion, in which the activity obtained was considerably lower and remained constant until birth. Extracts of whole

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TABLE 1. Expression of poly(A)$^+$ RNA coding for myeloid hematopoietic growth regulators in mouse fetal development

Source of RNA[a]	Expression			
	MGI-1M	MGI-1GM	MGI-1G	IL-3
Whole fetus	+	−	−	−
Placenta	++	−	−	−
Yolk sac plus amnion	+++	−	+++[b]	−
Lungs	+	−	−	−
Liver	+	−	−	−

[a] Poly(A)$^+$ RNAs were isolated from whole fetuses at 10, 13, 15, and 18 days of gestation, from placenta at 8, 10, 11, 13, 14, and 18 days of gestation, from yolk sac and amnion at 10, 13, 16, and 18 days of gestation, and from lungs and liver at 14 and 18 days of gestation.

[b] The transcript for MGI-1G was larger than the mature transcript of this gene.

fetuses and placentas contained little or no detectable MGI-1 activity (Fig. 4).

Since we detected MGI-1M-specific mRNA in placenta and fetuses but little or no protein activity, we attempted to ascertain whether the placental and fetal extracts contained inhibitors that interfered with our assay procedure. We compared extracts prepared by our extraction procedure (6) with extracts fractionated by ammonium sulfate precipitation (3). The results obtained were similar by both procedures (Table 2). Next, we performed mixing experiments, which showed that extracts of fetuses and placentas did not inhibit MGI-1 activity in amniotic fluid or extracts of fetal membranes (Table 2).

The MGI-1 activity in the amniotic fluid and yolk sac and amnion extracts induced the formation only of macrophage colonies. No mixed granulocyte-macrophage colonies or pure granulocyte colonies were observed, as in other experiments (10). The same results were obtained whether the bioassay was performed either with or without TPA (12-*O*-tetradecanoylphorbol-13-acetate) (14). This indicated that the MGI-1 activity was of the MGI-1M type. Further evidence for this was obtained by showing that an antiserum to L-cell MGI-1M (25) completely inhibited the MGI-1 activity in amniotic fluid, yolk sac, and amnion. To confirm that the MGI-1M activity was not due to lipopolysaccharide (LPS) induction (29), we tested amniotic fluid and fetal extracts from C3H/HeJ mice, which are resistant to LPS. The results obtained with C3H/HeJ mice were the same as with C3H/eBJ mice, which are susceptible to LPS.

No MGI-1GM-, MGI-1G-, or IL-3-specific activities were detected in amniotic fluid or fetal or extraembryonic tissue extracts. We therefore also tested cultures of fetal cells. Whole fetuses and yolk sac plus amnion cells were dissociated with trypsin-EDTA and seeded in tissue culture dishes to test for secretion of the specific factors into conditioned medium. We found that cultures of cells from whole fetuses from 9 to 15 days of gestation secreted 300 to 510 U of MGI-1M per ml. Conditioned medium from dissociated yolk sac and amnion cells in culture contained 520 to 680 U of MGI-1M per ml at 13 to 16 days of gestation. As in the fetus in vivo, the protein secreted by these cells in culture did not show any MGI-1GM, MGI-1G, or IL-3 activity.

Our present experiments were carried out to determine the temporal sequence of appearance and localization of myeloid hematopoietic cell growth regulators in the developing mouse fetus. Of the four myeloid growth regulators, only the growth inducer for macrophages (MGI-1M) was detected as both mRNA and bioactive protein in fetal development. At the mRNA level, MGI-1M-specific transcripts were found mainly in the fetal membranes and placenta and in lower amounts in the fetus itself. At the protein level, the overwhelming majority of extractable activity was confined to the yolk sac and amnion, which

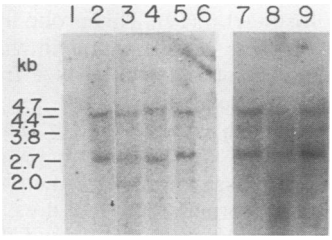

FIG. 1. Northern blot (RNA blot) analysis of mouse RNA hybridized to human MGI-1M cDNA. All lanes contain poly(A)$^+$ RNA, except lanes 1 and 6, which contain poly(A)$^-$ RNA as negative controls. Lanes: 1, L cells as negative control; 2, L cells as positive control; 3, yolk sac and amnion (day 10); 4, yolk sac and amnion (day 16); 5, yolk sac and amnion (day 18); 6, yolk sac and amnion, negative control (day 16); 7, placenta (day 9); 8, placenta (day 11); 9, placenta (day 16 of gestation).

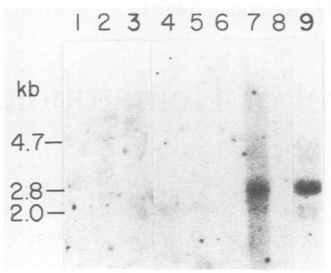

FIG. 2. Northern blot analysis of mouse RNA hybridized to human MGI-1G cDNA. All lanes contain poly(A)$^+$ RNA, except lane 8, which contains poly(A)$^-$ RNA as a negative control. Lanes: 1, WEHI cells; 2, L cells; 3, adult lungs activated with LPS; 4, day 10 placenta; 5, day 14 placenta; 6, day 18 placenta; 7, day 16 yolk sac and amnion; 8, day 16 yolk sac and amnion; 9, day 18 yolk sac and amnion.

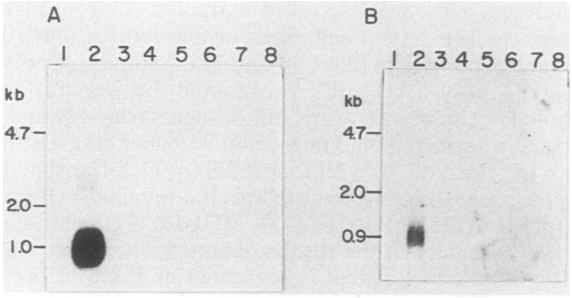

FIG. 3. Northern blot analysis of mouse RNA hybridized to mouse IL-3 cDNA (A) and mouse MGI-1GM cDNA (B). All lanes contain poly(A)$^+$ RNA, except lanes 1, which contain poly(A)$^-$ RNA from WEHI cells (A) or from adult lungs activated with LPS (B) as a negative control. (A) Lanes: 2, WEHI cells as a positive control; 3, day 10 placenta; 4, day 14 placenta; 5, day 18 placenta; 6, day 10 yolk sac and amnion; 7, day 16 yolk sac and amnion; 8, day 18 yolk sac and amnion. (B) Lanes: 2, adult lungs activated with LPS; 3, day 10 yolk sac and amnion; 4, day 16 yolk sac and amnion; 5, day 18 yolk sac and amnion; 6, day 10 placenta; 7, day 14 placenta; 8, day 18 placenta.

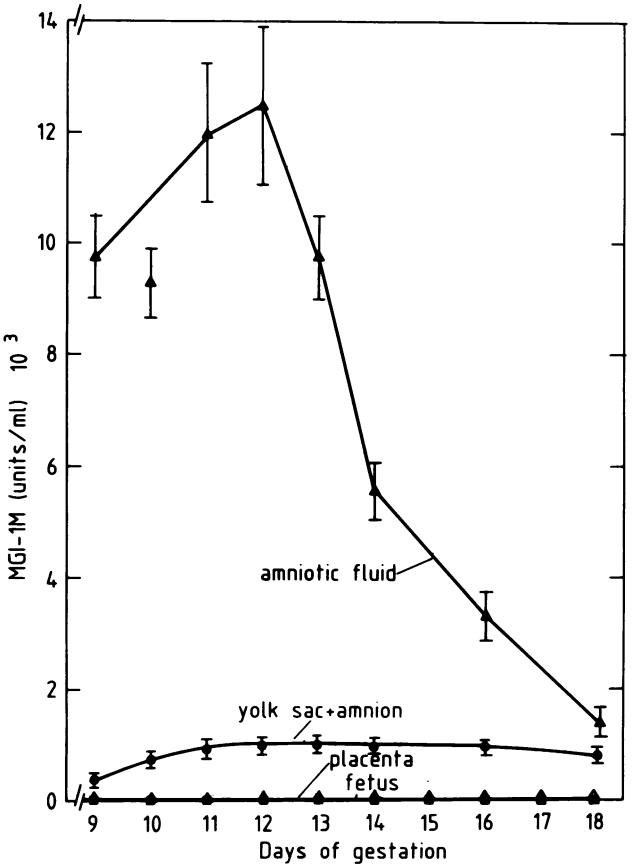

FIG. 4. MGI-1M protein activity in extracts of fetal and extraembryonic tissues and amniotic fluid of C3H/eBJ mice from 9 to 18 days of gestation. Samples were assayed (20) for the formation of colonies from normal mouse bone marrow cells. All the colonies (units) induced by these samples were macrophage colonies. Vertical bars indicate the standard error.

constitute the fetal membranes. The time course of appearance of MGI-1M protein in the amniotic fluid indicated that high levels of MGI-1M protein were already found at 9 days of gestation and that maximal activity was observed at 11 to 12 days of gestation. The early production of MGI-1M is presumably responsible for the early appearance of macrophage precursors in the embryo. At stages of embryogenesis showing high levels of MGI-1M protein, there was no detectable protein activity of a growth factor for granulocytes in the amniotic fluid or in extracts from fetal membranes, whole fetus, or placenta. Macrophages themselves can produce MGI-1GM (26), so that once these cells have differentiated, they could then also produce this inducer for macrophages and granulocytes.

Our results indicate that the production of specific myeloid growth regulators is strictly regulated during fetal development. The production of myeloid growth factors in vivo needs to be strictly controlled, both in sequence and in duration. The regulators are transcribed in a specific manner in particular tissues in development, and in some cases, such as MGI-1G, regulation may also occur at the posttranscriptional level. Interestingly, production in the extraembryonic membranes continued even at stages during which the fetal liver is the major hematopoietic organ. Thus, the regulators are apparently produced in a separate compartment from the target cells at these stages.

TABLE 2. MGI-1M protein activity in mixtures of amniotic fluid and extracts of fetal and extraembryonic tissues[a]

Materials tested	MGI-1M activity (U/ml)
Amniotic fluid	14,100 ± 1,250
Amniotic fluid + fetus extract	18,120 ± 1,400
Amniotic fluid + placenta extract	13,275 ± 975
Yolk sac and amnion extract	840 ± 60
Yolk sac and amnion extract + fetus extract	1,245 ± 120
Yolk sac and amnion extract + placenta extract	1,020 ± 75
Fetus extract	15 ± 10
Fetus extract—fractionated	37 ± 14
Placenta extract	24 ± 20
Placenta extract—fractionated	40 ± 10
Yolk sac and amnion extract—fractionated	993 ± 63

[a] Amniotic fluid (0.2%) or yolk sac and amnion extract (2%) were mixed with 5% fetus or placenta extract. These concentrations of amniotic fluid and yolk sac and amnion extract fell within the linear part of the activity curve. The mixed components were added together to the assay. U, Colonies. The amniotic fluid and tissues were taken from fetuses at 11 days of gestation. All extracts were prepared by freezing and thawing (6), except those denoted fractionated, which were prepared and partially purified by an ammonium sulfate procedure (3).

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