Critical windows in development of the rodent immune system

KS Landreth*

Department of Microbiology, Immunology, and Cell Biology and the Mary Babb Randolph Cancer Center, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, West Virginia 26505, USA

The immune system of rodents, like that in humans, develops from a population of pluripotent hematopoietic stem cells (HSC) that are generated early in gestation from uncommitted mesenchymal stem cells in the intraembryonic splanchnopleure surrounding the heart. This early population of HSC gives rise to all circulating blood cell lineages, including cells of the innate and acquired immune system. To access the impact of chemical exposure on the developing immune system and establish developmental windows of potential vulnerability to these exposures, it is essential to first consider the anatomical development of hematopoietic and lymphopoietic tissues and the sequence of appearance of cells that give rise to the immune system. This is particularly true in embryonic development because, after they initially appear in intraembryonic mesenchyme early in gestation, HSC migrate through an orderly series of tissues before establishing residence in the bone marrow and thymus. The effect of exposure to chemical insults in utero, then, may differ depending on the specific timing of exposure and anatomical location of hematopoiesis. Mechanisms and consequences of developmental immunotoxicity in experimental animals will need to be considered in that context. This review presents an overview of developmental hematopoiesis and a working hypothesis of critical developmental windows of vulnerability of this developmental system to toxic insult by chemical exposure. Human & Experimental Toxicology (2002) 21, 493–498.

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Development of the rodent immune system in utero and in postnatal life is a dynamic process that involves continual differentiation of lineage-restricted stem cells for myeloid (innate immune) or lymphoid (acquired immune) leukocytes from a set of pluripotential hematopoietic stem cells (HSC). These lineage-restricted stem cells expand to form a pool of highly proliferative progenitor cells that are capable of continual renewal of short-lived functional immunocompetent cells. This rapid renewal of immunocompetent cells provides the necessary cellular capacity for effective innate immune responsiveness and the necessary breadth of immune repertoire required for effective acquired immune responses. Failure to establish either innate cellular capacity or a pool of immune clonotypes required for effective lymphocyte clonal selection results in immunodeficiency.1

Establishment of these populations of lymphoid-hematopoietic progenitor cells in the mammalian embryo takes place in a punctuated temporal sequence of events that involves emergence of hematopoietic cells in developing intraembryonic tissues, migration of these cells from intraembryonic mesenchyme to fetal liver and fetal spleen, and ultimately relocation of these cells to bone marrow and thymus in late gestation.2 The bone marrow and thymus appear to be unique in providing microenvironment factors necessary for development of functionally immunocompetent cells.3–6 This well-documented series of developmental events has resulted in a working hypothesis that a series of critical windows of vulnerability to chemical exposure exist for the developing immune system, and that these critical windows may be differentially susceptible to chemical exposure.7 This hypothesis is currently being tested and will need to be considered in designing experimental protocols to determine acute and persistent immunotoxic effects of environmentally applied chemicals. It is also important to realize that renewal of immunocompetent cells in postnatal animals continues to depend on differentiation of stem cells and expansion of progenitor cells in the bone marrow, and that this process is fundamentally different in neonatal, young, and older animals. For that reason, developmental windows of vulnerability to chemical exposure are not restricted to the period of prenatal development.

*Correspondence: Kenneth S. Landreth, PhD, Mary Babb Randolph Cancer Center, P.O. Box 9300, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, West Virginia 26505, USA.
E-mail: klandreth@wvu.edu

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Our current understanding of development of the immune system in rodents has come almost exclusively from mechanistic experiments in mice. For that reason, mouse developmental immunology will largely be the focus of the information presented in this summary. We know far less about the development of immunocompetent cells in rats; however, the general outline and timing of immune cell development in both rats and humans appears to closely parallel studies in mice where data are available.

**Emergence of HSC in development**

HSC are a population of multipotential stem cells that retain the capacity to self-renew and which have the capacity to differentiate to form all subclasses of leukocytes that participate in immune responses, as well as megakaryocytic and erythroid cells. HSC first appear developmentally in intraembryonic splanchnopleuric mesenchyme surrounding the heart (or the aorta-gonadomesonephros, AGM) at approximately eight days of gestation in mice (Figure 1). These cells are found at essentially the same developmental stage in the extraembryonic blood islands of the yolk sac. Embryonic circulation is established by gestational day 8.5 in the mouse and it remains unclear to what extent there is exchange of cells from intraembryonic hematopoietic tissues to extraembryonic sites in any rodent species. However, recent evidence clearly demonstrates that the population of intraembryonic stem cells, but not those which appear in the yolk sac, contribute to sustained intraembryonic blood cell development and emergence of the immune system in postnatal rodents. This initial period of commitment of mesenchymal stem cells to hematopoietic cell development culminates with the migration of HSC to new intraembryonic anatomic locations that support their further differentiation. Differentiation of HSC to form lineage-restricted subpopulations of stem cells for the lymphoid and myeloid cell lineages has not been demonstrated before gestational day 10 in mice.

**Fetal liver hematopoiesis**

HSC migrate from the anterior gonadomesonephric region (AGM) of the embryo to the developing fetal liver by gestation day 10 in mice. In the fetal liver, HSC differentiate and form a cascade of progressively more differentiated and restricted stem cells and progenitor cells which are defined by their potential to form colonies in vitro in response to specific cytokine stimulation (Figure 1). CFU are defined by the specific cytokine that stimulates their formation and the predominant cell type that populate the colony. This method of functionally defining progenitor cell population is operationally more useful for studying early events in hematopoiesis than classification schemes based on cell phenotype because phenotypically recognizable cells are developmentally later and largely postmitotic cell forms.

The fetal liver continues as the primary hematopoietic tissue throughout gestation and is characterized by emergence and rapid expansion of lineage-restricted progenitor cells for all types of leukocytes. For this reason, mechanistic studies of normal and disrupted rodent developmental hematopoiesis and lymphopoiesis have focused on this tissue. Likewise, mechanistic studies of developmental effects of chemical exposure will necessarily need to consider toxic effects on development of cells in this embryonic tissue.

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**Figure 1** Hematopoiesis. HSC and lineage-restricted cells are depicted with an indication of the colony forming unit (CFU) assays that are used to enumerate each cell type experimentally.

**Figure 2** Embryonic development of hematopoiesis. The duration of residence of hematopoietic cells in embryonic tissues is indicated by gestational day.
Interestingly, the period of rapid hematopoietic progenitor cell expansion in fetal liver is not accompanied by wholesale differentiation to mature immunocompetent cell phenotype. In fact, mature lymphocytes are not found in the developing liver until day 18 of gestation in the mouse, and after the initiation of hematopoiesis in embryonic bone marrow.

**Splenic hematopoiesis**

HSC and lineage-restricted hematopoietic progenitor cells are found in fetal spleen from approximately gestational day 13 (Figure 2). The spleen continues to contain limited reserves of hematopoietic cells into postnatal life and support myeloid and erythroid cell development, particularly if the bone marrow is damaged. However, lymphopoiesis does not occur in the spleen of postnatal mice under any experimental conditions tested. These latter observations have led to the hypothesis that the bone marrow hematopoietic microenvironment is unique and required for lymphocyte production in mice.

**Thymus development**

In mice, the thymus anlage develops from the third and fourth pharyngeal pouches on gestational day 10 and is colonized by immigrating HSC by gestational day 11 (Figure 2). Pluripotent HSC continue to be detectable in the thymus throughout gestation; however, the majority of immigrating cells differentiate within the thymic microenvironment to form immature proliferating lymphoid thymocytes which express the T cell specific surface proteins, CD3, CD4, CD8 and the T cell receptor. As with other hematopoietic cells, immature proliferating thymocytes form colonies in vitro when exposed to the proliferative cytokines IL-7 and ckit-ligand (CFU-IL-7). Developing thymic lymphocytes initiate expression of the T cell receptor (TcR), undergo a series of selection events which remove autoreactive cells and ultimately migrate out of that tissue expressing TcR and a set of lineage specific membrane glycoproteins that define phenotype and predict function of these cells. Thymic cell production wanes rapidly following sexual maturity in all vertebrates, ultimately resulting in virtual absence of thymocyte production at one year of life in mice.

**Establishment of bone marrow as a hematopoietic organ**

The final critical stage in embryonic development of hematopoiesis that is essential to development of the immune system is formation of the bone marrow cavity as long bones are mineralized late in gestation and immigration of hematopoietic cells into that tissue site on gestational day 17.5 in mice. The relationship of bone formation and emergence of hematopoietic tissue is particularly interesting, and it is unclear whether the migration of hematopoietic cells into this tissue actually initiates the process of bone ossification. The evacuated bone marrow cavity is colonized by HSC derived from cells that originated in the AGM and these pluripotent cells expand to establish the primary hematopoietic reserves of stem cells for postnatal life. It is less clear whether lineage-restricted stem and progenitor cells produced in the fetal liver also migrate to the bone marrow along with stem cells. The bone marrow rapidly assumes primary hematopoietic function after embryonic day 18 in mice and this persists throughout postnatal life.

**Critical windows of vulnerability in fetal development of the immune system**

As shown in Figure 3, these data can be used to propose three distinct windows of vulnerability of the developing murine hematopoietic system to toxic insult. Gestational days 7.5–10.5 encompass a period of HSC formation from undifferentiated mesenchymal cells. Exposure of the embryo to toxic chemicals during this period could result in failures of stem cell formation, abnormalities in production of all hematopoietic lineages, and immune failure. Gestational days 10.5–16.5 are characterized by migration of hematopoietic cells to the fetal liver and thymus, differentiation of lineage-restricted stem cells, and expansion of progenitor cells for each leukocyte

![Figure 3 Critical windows of vulnerability during embryonic development of the immune system](image)
lineage. This developmental window is likely to be particularly sensitive to agents that interrupt cell migration, adhesion, and proliferation.

The primary developmental event in from gestational day 17 to birth is the establishment of bone marrow as the primary hematopoietic site. This developmental milestone requires migration of stem cells, calcification of the cartilaginous skeletal elements, and remodeling of ossified tissue. In addition, the establishment of the bone marrow hematopoietic microenvironment appears to be a unique process that is essential for normal postnatal production of lymphocytes, neutrophils, and monocytes. This set of developmental process that result in steady-state postnatal hematopoiesis may be uniquely susceptible to chemical toxicities.

**Postnatal development of the immune system**

Following birth, there is an immediate disappearance of hepatic and splenic hematopoietic function as embryonic organs assume postnatal functions. In the postnatal mouse, the liver is devoid of hematopoietic function and leukocytes are produced in the bone marrow and, except for T lymphocytes, complete their maturation in that tissue. Mature leukocytes exit the bone marrow by transiting through endothelial cell membranes, a process known as pseudoinperipoiesis.

It is during the immediate postnatal period that acquired immune function is first detectable in mammals (Figure 4). Functional B and T lymphocytes are produced in the bone marrow and thymus, respectively, and migrate to the spleen, lymph nodes, and mucosal associated lymphoid tissues (MALT).

However, a mature pattern of immune response to antigen is not achieved until approximately one month of age in rodents. During the first month of postnatal life in mice and the first year in humans, the immune system remains immature, fails to produce antibodies to carbohydrate antigens, and is predominated by IgM-mediated immune responses to antigen. For that reason, evaluation of mature immune responses in rodents must be evaluated after this initial period of perinatal immunodeficiency (Figure 4).

**Establishment of immune memory**

During the first six months of life in rodents, there is enormous production of T and B lymphocytes from primary lymphoid tissues. Exposure to specific antigen during this period results in a rapidly expanding accumulation of lymphocyte specificities in the pool of memory cells in secondary lymphoid tissues. As thymic function wanes and thymocytes are no longer produced in that tissue, it is this pool of memory B and T cells that maintains immunocompetence for the life of the individual.

Senescence of the immune responses is not well understood, but it is clear that both innate and acquired immune responses to antigens are different in rodents in the last quarter of their life. This failure of the immune response is due, in part, to failure of production of newly formed cells and decreased survival of long-lived cells in lymphoid tissues.

**Hematopoietic microenvironment**

The microenvironment of the bone marrow has been the focus of considerable attention in recent years. Fibroblastic bone marrow stromal cells produce most of the cytokines that regulate hematopoiesis and are required for establishing long-term bone marrow cultures in vitro. Absence or failure of stromal cell function is known to result in failure of hematopoietic cells to survive, proliferate, or differentiate to form functional immunocompetent end cells. A role for resident bone marrow macrophages and endothelial cells in regulating hematopoiesis has also been proposed.

The regulatory role proposed for the bone marrow hematopoietic microenvironment in maintaining the continual production of leukocytes required for sustained immunocompetence has led to the proposal that exposure to toxic chemical may affect production of hematolymphoid cells by damaging the stromal microenvironment. This form of indirect hematotoxicity is intriguing and currently under investigation in a number of laboratories.
Critical windows of exposure for the developing hematopoietic system

This understanding of the developmental progression of cells and tissues of the immature immune system has allowed construction of a logical experimental model for determining developmental immunotoxicity of chemical compounds (Figure 5). This model is segmented into five critical windows of potential vulnerability. In the embryo, these are the period of embryonic stem cell formation, the period fetal liver development as the primary embryonic hematopoietic organ, and the period of colonization and establishment of the bone marrow and thymus as the ultimate hematopoietic organs during the final week of gestation. In the postnatal rodent, the critical periods of immune system development are the initial period of perinatal immunodeficiency as the immune system matures to adult function, and the subsequent period during which mature immune responses take place and establish a functional pool of protective memory cells.

These five windows of embryonic vulnerability to damage by environmental exposures also define the formation and function of both hematopoietic tissues and secondary lymphoid tissues that may have differential sensitivity to specific exposures (Figure 4). In the embryo, stem cells are initially formed in the AGM region, progenitor cell expansion and differentiation takes place primarily in the fetal liver, the final stage of maturation of precursors to immunocompetent cells occurs in the bone marrow and thymus. Mechanistic studies which aim to better understand acute toxicity to the developing immune system during gestation should limit chemical exposures to these specific developmental windows of vulnerability and evaluate acute toxicity in appropriate tissue sites based on this model.

As noted earlier, immune system development does not cease at birth, and immunocompetent cells are identified in yet another set of anatomic locations in postnatal rodents (Figure 4). Myeloid and lymphoid cells continue to be produced from proliferating progenitor cells in the bone marrow and thymus. Mature immunocompetent cells leave these central hematopoietic organs and migrate via the blood to the secondary immune organs, the spleen, lymph nodes, and mucosal lymphoid tissues. In addition, plasma cells, the antibody-producing end cells of the B lymphoid compartment, take up residence in the bone marrow and produce substantial quantities of antibody in that tissue. For that reason, the bone marrow is the primary anatomic location of both newly formed and fully mature B lymphoid cells.

Understanding the effect of immunotoxic compounds on these postnatal immune responsive tissues is complicated by the fact that the same cells may have differential susceptibility to toxic compounds depending on their anatomic location (microenvironment) at the time of exposure. In addition, resident cells in any of these tissues may have experienced toxic damage in other anatomic locations and then migrated to a different tissue site when they are assayed.

Cell renewal and repopulation of lymphoid tissues

The hematopoietic and immune systems are unique in their ability to recover from toxic insult. Numerous studies have demonstrated rapid recovery of immune responsive cells following cytotoxic drug therapy and the ability of hematopoietic cells to recover following depletion is sufficiently robust to justify clinical utility of bone marrow transplantation. For that reason, relatively dramatic acute toxicity to either blood cell development or immune cell function may not lead to persistent detection of immunotoxicity. However, underlying damage to stem and progenitor cell populations may not be detected by routine studies that enumerate numbers of cells that respond to a single antigen and investigators may need to develop a battery of hematopoietic assays to accurately catalog the long-term effects of toxic chemical exposure on the blood-forming system.

References


