



**Defense Threat Reduction Agency**  
8725 John J. Kingman Road, MS  
6201 Fort Belvoir, VA 22060-6201



**DTRA-TR-12-16**

# TECHNICAL REPORT

## **Hematopoiesis Primer Modeling Combined Injury**

Approved for public release; distribution is unlimited.

May 2012

DTRA01-03-D0014

Terry C. Pellmar et al.

Prepared by:  
ARA, Inc.  
801 N. Quincy Stret  
Suite 700  
Arlington, VA 22203

DESTRUCTION NOTICE

Destroy this report when it is no longer needed.  
Do not return to sender.

PLEASE NOTIFY THE DEFENSE THREAT REDUCTION  
AGENCY, ATTN: OP-ONIUI, 8725 JOHN J. KINGMAN ROAD,  
MS-6201, FT BELVOIR, VA 22060-6201, IF YOUR ADDRESS  
IS INCORRECT, IF YOU WISH IT DELETED FROM THE  
DISTRIBUTION LIST, OR IF THE ADDRESSEE IS NO  
LONGER EMPLOYED BY YOUR ORGANIZATION.

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                               |                                           |                                       |                                                             |                                                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|-------------------------------------------|---------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------------------|
| <b>REPORT DOCUMENTATION PAGE</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                               |                                           |                                       | <i>Form Approved</i><br><b>OMB No. 0704-0188</b>            |                                                                         |
| Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.<br><b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>                                    |                               |                                           |                                       |                                                             |                                                                         |
| <b>1. REPORT DATE (DD-MM-YYYY)</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                               | <b>2. REPORT TYPE</b><br>Technical Report |                                       | <b>3. DATES COVERED (From - To)</b><br>December 2010- 2012  |                                                                         |
| <b>4. TITLE AND SUBTITLE</b><br>Hematopoiesis Primer for Modeling Combined Injury                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                               |                                           |                                       | <b>5a. CONTRACT NUMBER</b><br>DTRA01-03-D-0014-0038         |                                                                         |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                               |                                           |                                       | <b>5b. GRANT NUMBER</b>                                     |                                                                         |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                               |                                           |                                       | <b>5c. PROGRAM ELEMENT NUMBER</b>                           |                                                                         |
| <b>6. AUTHOR(S)</b><br>Terry C. Pellmar and Glen I. Reeves                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                               |                                           |                                       | <b>5d. PROJECT NUMBER</b>                                   |                                                                         |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                               |                                           |                                       | <b>5e. TASK NUMBER</b>                                      |                                                                         |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                               |                                           |                                       | <b>5f. WORK UNIT NUMBER</b>                                 |                                                                         |
| <b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b><br>Applied Research Associates,<br>Inc.<br>801 N. Quincy Street, Ste. 700<br>Arlington, VA 22203                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                               |                                           |                                       | <b>8. PERFORMING ORGANIZATION<br/>REPORT NUMBER</b>         |                                                                         |
| <b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b><br><br>Defense Threat Reduction Agency<br>8725 John J. Kingman Road, MSC<br>Fort Belvoir, VA 22060                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                               |                                           |                                       | <b>10. SPONSOR/MONITOR'S<br/>ACRONYM(S)</b><br>DTRA/RD-NTSN |                                                                         |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                               |                                           |                                       | <b>11. SPONSOR/MONITOR'S REPORT<br/>NUMBER(S)</b>           |                                                                         |
| <b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b><br>Approved for public release; distribution is unlimited.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                               |                                           |                                       |                                                             |                                                                         |
| <b>13. SUPPLEMENTARY NOTES</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                               |                                           |                                       |                                                             |                                                                         |
| <b>14. ABSTRACT</b><br><br>This report is an overview of the basic principles underlying the formation, function, and response to injury of the different elements of the hematopoietic system. The adverse effects of radiation on the hematopoietic system differ from those of thermal burn or traumatic wounding. In turn, the effects of burn injury and trauma with hemorrhage differ from one another. Accordingly, the effects of individual injurious modality on each hematopoietic element are considered. The cell kinetics of myelopoiesis under irradiation are used in the current model of Radiation-Induced Performance Decrement (RIPD) to estimate lethality. This report is also intended to highlight potential roles for hematopoietic effects in the new models of RIPD to incorporate findings in recent assessments of changes of blood cells in combined injury models (combined injury being defined as radiation injury plus burn or wound trauma injury, or both). |                               |                                           |                                       |                                                             |                                                                         |
| <b>15. SUBJECT TERMS</b><br>Combined injury      Modeling      Hematopoiesis      RIPD model                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                               |                                           |                                       |                                                             |                                                                         |
| <b>16. SECURITY CLASSIFICATION OF:</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                               |                                           | <b>17. LIMITATION<br/>OF ABSTRACT</b> | <b>18. NUMBER<br/>(of pages)</b>                            | <b>19a. NAME OF RESPONSIBLE<br/>PERSON</b><br>Dr. Paul K. Blake         |
| <b>a. REPORT</b><br>UNCLASS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | <b>b. ABSTRACT</b><br>UNCLASS | <b>a. THIS PAGE</b><br>UNCLASS            | Unlimited                             | 34                                                          | <b>19b. TELEPHONE NUMBER</b><br>(include area code)<br><br>703-767-3384 |

# CONVERSION TABLE

## Conversion factors for U.S. customary to metric (SI units of measurement)

| MULTIPLY<br>TO GET                               | BY<br>BY             | TO GET<br>DIVIDE                                   |
|--------------------------------------------------|----------------------|----------------------------------------------------|
| angstrom                                         | 1.000 000 x E-10     | meters (m)                                         |
| atmosphere                                       | 1.012 25 x E +2      | kilo pascal (kPa)                                  |
| bar                                              | 1.000 000 x E + 2    | kilo pascal (kPa)                                  |
| barn                                             | 1.000 x E – 28       | meter <sup>2</sup> (m <sup>2</sup> )               |
| British thermal unit (thermochemical)            | 1.054 350 x E + 3    | joule (J)                                          |
| calorie (thermochemical)                         | 4.184 000            | joule (J)                                          |
| cal (thermochemical)/cm <sup>2</sup>             | 4.184 000 x E-2      | mega joule/m <sup>2</sup> (MJ/m <sup>2</sup> )     |
| curie                                            | 3.7000 000 x E + 1   | giga becquerel (GBq)*                              |
| degree (angle)                                   | 1.745 329 x E – 2    | radian (rad)                                       |
| degree (Fahrenheit)                              | Tk = (t +459.69)/1.8 | degree kelvin (K)                                  |
| electron volt                                    | 1.602 19 x E – 19    | joule (J)                                          |
| erg                                              | 1.000 000 x E – 7    | joule (J)                                          |
| erg/sec                                          | 1.000 000 x E – 7    | watt (W)                                           |
| foot                                             | 3.048 000 x X-1      | meter (m)                                          |
| foot-pound-force                                 | 1.355 818            | joule (J)                                          |
| gallon (U.S. liquid)                             | 3.785 412 x E – 3    | meter <sup>3</sup> (m <sup>3</sup> )               |
| inch                                             | 2.540 000 x E -2     | meter (m)                                          |
| jerk                                             | 1.000 000 x E + 9    | joule (J)                                          |
| joule/kilogram (J/kg) (absorbed dose)            | 1.000 000            | Gray (Gy)**                                        |
| kilotons                                         | 4.183                | terajoules                                         |
| kip (1000 lbf)                                   | 4.448 222 x E + 3    | newton (N)                                         |
| kip/inch <sup>2</sup> (ksi)                      | 6.894 757 x E +3     | kilo pascal (kPa)                                  |
| ktap                                             | 1.000 000 x E +2     | newton-second/m <sup>2</sup> (N-s/m <sup>2</sup> ) |
| micron                                           | 1.000 000 x E – 6    | meter (m)                                          |
| mil                                              | 2.540 000 x E – 5    | meter (m)                                          |
| mile (international)                             | 1.609 344 x E + 3    | meter (m)                                          |
| ounce                                            | 2.834 952 x E – 2    | kilogram (kg)                                      |
| pound-force (lbf avoirdupois)                    | 4.448 222            | newton (N)                                         |
| pound-force inch                                 | 1.129 848 x E – 1    | newton-meter (N*m)                                 |
| pound-force/inch                                 | 1.751 268 x E + 2    | newton-meter (N/m)                                 |
| pound-force/foot <sup>2</sup>                    | 4.788 026 x E – 2    | kilo pascal (kPa)                                  |
| pound-force/inch <sup>2</sup> (psi)              | 6.894 757            | kilo pascal (kPa)                                  |
| pound-mass-foot <sup>2</sup> (moment of inertia) | 4.214 011 x E – 2    | kilogram-meter <sup>2</sup> (kg*m <sup>2</sup> )   |
| pound-mass/foot <sup>3</sup>                     | 1.601 846 x E + 1    | kilogram/m <sup>3</sup> (kg/m <sup>3</sup> )       |
| rad (radiation absorbed dose)                    | 1.000 000 x E – 2    | Gray (Gy) **                                       |
| rem (roentgen equivalent man)                    |                      | Sievert (Sv) ***                                   |
| roentgen                                         | 2.579 760 x E – 4    | coulomb/kilogram (C/kg)                            |
| shake                                            | 1.000 000 x E – 8    | second (s)                                         |
| Slug                                             | 1.459 390 x E + 1    | kilogram (kg)                                      |
| Torr (mm Hg, 0 degrees C)                        | 1 333 22 x E – 1     | kilo pascal (kPa)                                  |

\* The Becquerel (Bq) is the SI unit of radioactivity: 1 Bq = 1 event/s.

\*\* The Gray (Gy) is the SI unit of absorbed radiation.

\*\*\* The Sievert (SV) is the SI unit of dose equivalent

## TABLE OF CONTENTS

|                                        |                   |
|----------------------------------------|-------------------|
| CONVERSION TABLE .....                 | iii               |
| LIST OF FIGURES .....                  | <a href="#">v</a> |
| 1.0 INTRODUCTION .....                 | 1                 |
| 2.0 HEMATIC CELLS .....                | 2                 |
| 2.1 LYMPHOCYTES .....                  | 2                 |
| 2.1.1 Lymphocyte Functions .....       | 2                 |
| 2.1.2 Lymphopoiesis .....              | 2                 |
| 2.1.3 Radiation Effects .....          | 4                 |
| 2.1.4 Trauma Effects .....             | 4                 |
| 2.1.5 Role in RIPD and CI Models ..... | 5                 |
| 2.2 GRANULOCYTES .....                 | 5                 |
| 2.2.1 Granulocyte Functions .....      | 5                 |
| 2.2.2 Granulopoiesis .....             | 6                 |
| 2.2.3 Radiation Effects .....          | 7                 |
| 2.2.4 Trauma Effects .....             | 7                 |
| 2.2.5 Role in RIPD and CI Models ..... | 8                 |
| 2.3 THROMBOCYTES .....                 | 9                 |
| 2.3.1 Thrombocyte Functions .....      | 9                 |
| 2.3.2 Thrombopoiesis .....             | 9                 |
| 2.3.3 Radiation Effects .....          | 10                |
| 2.3.4 Trauma Effects .....             | 10                |
| 2.3.5 Role in RIPD and CI Models ..... | 11                |
| 2.4 ERYTHROCYTES .....                 | 12                |
| 2.4.1 Erythrocyte Functions .....      | 12                |
| 2.4.2 Erythropoiesis .....             | 12                |
| 2.4.3 Radiation Effects .....          | 13                |
| 2.4.4 Trauma Effects .....             | 13                |
| 2.4.5 Role in RIPD and CI Models ..... | 13                |
| 3.0 SUMMARY .....                      | 14                |
| 4.0 REFERENCES .....                   | 15                |
| APPENDIX A. ACRONYMS .....             | A-1               |
| APPENDIX B. GLOSSARY .....             | B-1               |
| APPENDIX C. DISTRIBUTION LIST .....    | C-1               |

## LIST OF FIGURES

|                                                        |    |
|--------------------------------------------------------|----|
| Figure 1 Hematopoietic elements in humans.....         | 3  |
| Figure 2. Lymphopoietic component.....                 | 4  |
| Figure 3. Granulocytic component plus macrophage ..... | 7  |
| Figure 4. Thrombopoietic component.....                | 10 |
| Figure 5. Erythrocytic component .....                 | 12 |

## 1.0 INTRODUCTION

Changes in circulating blood cells contribute significantly to acute morbidity and mortality from radiation injury; therefore mathematical models of the effects of radiation must include models of the specifics of hematopoiesis, the production of new blood cells (Gk. *haima*, blood; *poiesis*, a making). This report is an overview of the basic principles underlying the formation, function, and response to radiation, thermal burn and traumatic injury of the different elements of the hematopoietic system. The adverse effects of radiation on the hematopoietic system differ from those of thermal burn or traumatic wounding. In turn, the effects of burn injury and trauma with hemorrhage differ from one another (Lederer *et al.* 2007). Accordingly, the effects of individual injurious modalities on each hematopoietic element are considered. The report also describes the importance each of the blood lines may have in modeling combined injury (CI), the combination of radiation plus burn or trauma.

Physiologically based models of radiation injury form the basis of the Radiation-Induced Performance Decrement (RIPD) software tool. RIPD estimates lethality by calculating the cell kinetics of myelopoiesis under irradiation, with either prompt or protracted radiation exposure (Jones *et al.* 1994). The model assumes that the surviving fraction of at least one population of cells determines the probability of hematopoietic lethality. The specific cell that constitutes the population critical to hematopoietic lethality is not specified in the model. RIPD also uses a model of lymphopoiesis (Zukhbaya and Smirnova 1991) to describe changes in the lymphocyte population after radiation exposure and to predict consequent fatigability and weakness. Models currently in development for radiation and combined injury are incorporating a more detailed assessment of changes of the blood cells with radiation and/or trauma. The various hematopoietic cells contribute to a variety of physiological and pathological processes and are integral to common pathways for the effects of radiation and traumatic injuries. Some of the potential roles for hematopoietic effects in the new models are highlighted in this report.

## **2.0 HEMATIC CELLS**

Within the red bone marrow of healthy adults, a common, pluripotent hematopoietic stem cell gives rise to all of the blood cells; this is shown graphically in Figure 1. This stem cell divides and differentiates into common myeloid progenitor cells or common lymphoid progenitor cells. The myeloid (Gk. *myelo*, marrow) progenitor cell differentiates further to produce granulocytes and monocytes including macrophages, thrombocytes, and erythrocytes. The lymphoid progenitor gives rise to the various types of lymphocytes. Within the bone, hematopoietic stem cells and progenitor cells are localized to specific niches with different micro-environments that influence their regeneration, differentiation, and mobilization. Local osteoblasts (bone tissue precursors), endothelial cells, and other cells in the bone marrow niches contribute to these microenvironments (Pellmar 2011).

The remainder of this section describes the basic principles underlying the formation, function, and response to injury of the different elements of the hematopoietic system. In addition, the impact that radiation, burn and trauma insults have on each specific blood cell line is described. Finally, where appropriate, the role of each blood cell line has in RIPD or combined injury (CI) modeling is also discussed.

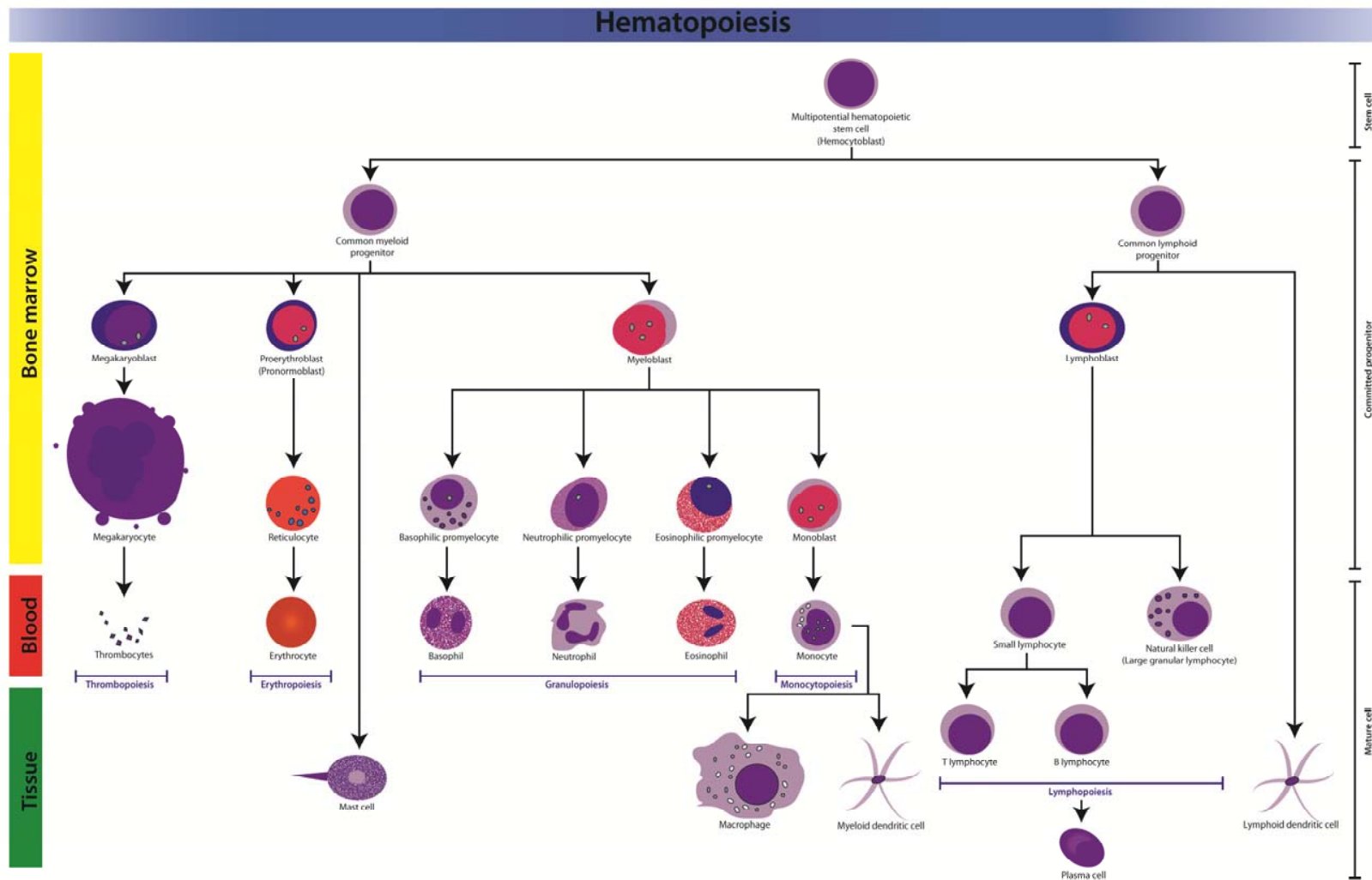
### **2.1 LYMPHOCYTES**

#### **2.1.1 Lymphocyte Functions**

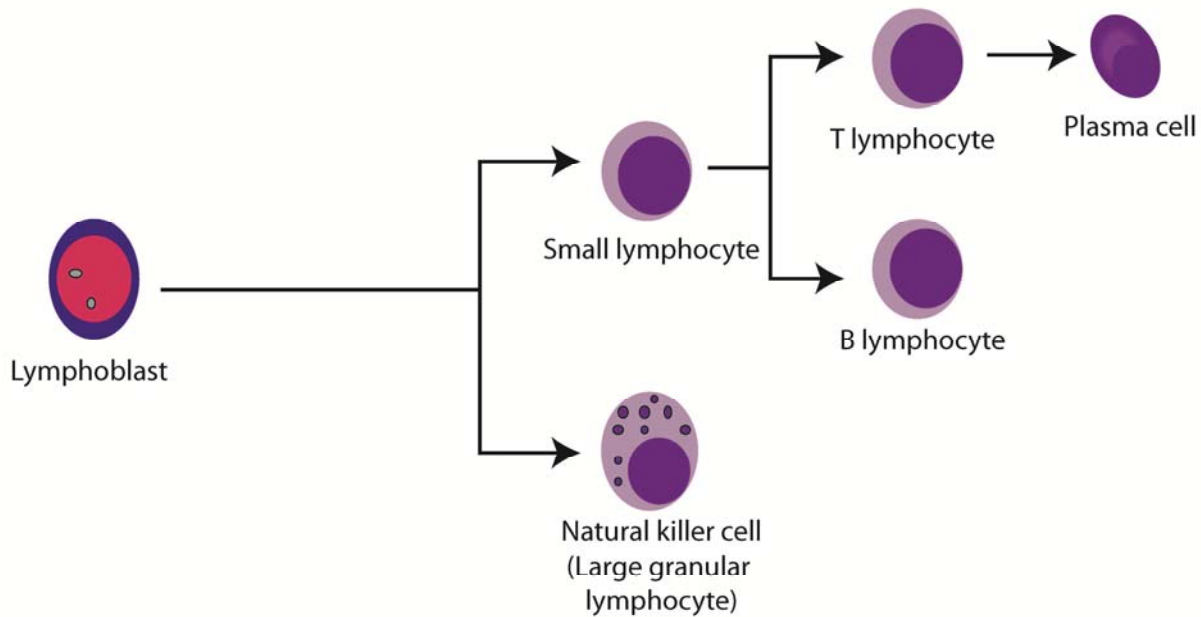
Lymphocytes are a class of white blood cells (or leukocytes) involved in the body's immune response. There are two major classes of lymphocytes, B cells and T cells, plus other subpopulations including natural killer (NK) cells. Several subcategories of T cells exist, including helper, cytotoxic, memory, and regulatory cells. T cells contribute to the immune response in a variety of ways including cell-mediated antigen recognition, release of cytokines, and suppression of B cells. B cells, which comprise 30% of circulating lymphocytes, are responsible for antibody production. Interaction of the B cell with an appropriate CD4<sup>+</sup> helper T cell is required for this process.

#### **2.1.2 Lymphopoiesis**

A common lymphoid progenitor in the bone marrow gives rise to lymphoblasts, which undergo several mitotic divisions to become prolymphocytes as shown in Figure 2. These prolymphocytes have specific cell-surface markers that are unique to either B or T cells. Although both T and B lymphocytes originate in the bone marrow, T cells migrate to the thymus for maturation. Proliferation and differentiation are influenced by numerous signals from the microenvironment, including cytokines and growth factors. Once mature, the lymphocytes enter the bloodstream but recirculate through the various lymphoid tissues (i.e., lymph nodes, spleen, thymus, etc.).



**Figure 1 Hematopoietic elements in humans**



**Figure 2. Lymphopoietic component**

### 2.1.3 Radiation Effects

Circulating lymphocytes are very sensitive to radiation exposure. Lymphopenia is one of the first signs of radiation exposure. The lymphocyte counts drop quickly within the first 24 hours post-irradiation and reach a minimum within 48 hours. The magnitude and rate of the effect is dose dependent. Because this response is very robust, the time course of lymphocyte depletion is an excellent measure of exposure and is likely to be used for biodosimetric assessments. Not all lymphocytes are equally sensitive to radiation however. At least one subpopulation of T cells shows a relative increase in numbers after exposure (e.g. see Nagayama *et al.* 2002, Qu *et al.* 2010).

### 2.1.4 Trauma Effects

Burn affects the production and function of lymphocytes with differential effects on the subpopulations. Some studies (e.g. Schlüter *et al.* 1990) report that B cells in circulation are unchanged, but Fayazov *et al.* (2009) and Jarvis (2007) found that the number in circulation after a burn more than doubled. T cells, particularly T-helper cells, in circulation seem to decrease slightly (Fayazov *et al.* 2009, Burleson *et al.* 1987) but this observation may be impacted by the incidence of infection. Dysfunction of T cells may contribute to the inflammatory response that results from burns (Barlow 1994, Schwacha 2009). In addition, down-regulation of human leukocyte antigen expression on B and T lymphocytes, as well as monocytes, has been detected early after trauma (Flohé *et al.* 2003, Ditschkowski *et al.* 1999); this may render patients particularly vulnerable to sepsis (Flohé *et al.* 2003).

Shock-related immunosuppression has been noted after trauma and hemorrhage and has been implicated in immunologic derangement and development of multiple organ dysfunction

syndrome (MODS). In trauma and hemorrhagic shock, the balance between cell proliferation and cell death is perturbed. Parreira *et al.* (2004) reported increased bone marrow apoptosis after hemorrhagic shock. The increase in apoptotic cells is not the result of deficient phagocytosis but factors released by the injury.

### **2.1.5 Role in RIPD and CI Models**

The Fatigability and Weakness model within RIPD uses Smirnova's model of lymphopoiesis (Zukhbaya 1991) to calculate the population and kinetics of lymphocytes and their resulting cytokine production after radiation. In Smirnova's model, the total lymphocyte population is comprised of three subpopulations or compartments: a dividing progenitor cell ( $X_1$ ), a nondividing intermediate cell ( $X_2$ ), and a mature circulating cell ( $X_3$ ). Each of these compartments expresses a chalone, or mitotic inhibitor, that controls the rate of proliferation of the  $X_1$  cells and consequently the cell populations of all three compartments. After irradiation the  $X_1$ ,  $X_2$ , and  $X_3$  cells can become damaged or heavily damaged, resulting in different rates of chalone production. The model assumes that the damaged lymphocytes serve as a source of cytokines that reflect the symptoms of fatigability. Recent data support a link between specific cytokines and fatigability and weakness after radiation therapy (Bower *et al.* 2009). Symptoms of fatigue are associated with increases in certain subpopulations of T cell lymphocytes (e.g., Bower *et al.* 2003, Lorusso *et al.* 2009). A role for T cells in fatigability is possible since some T cell subpopulations that are relatively resistant to radiation injury persist post-exposure (e.g., Qu *et al.* 2002). These findings suggest that fatigability and weakness may be related to cytokine release from persistent functioning T cells, rather than cytokines released from damaged lymphocytes (Pellmar and Oldson 2011).

In addition to prediction of fatigability, calculations of the lymphocyte population with radiation and/or trauma exposure are likely to be valuable in modeling combined injury. The mechanisms used by the immune cells to combat infection contribute to an inflammatory response (Chaplin 2010). For example, B cells are a source of a variety of both inflammatory and anti-inflammatory cytokines including IL-6, IL-8 and IL-10. Although lymphocytes contribute smaller quantities of these messengers compared to granulocytes, they are likely to contribute to the total response (Nikolajczyk 2010). Changes in the lymphocyte population, therefore, can impact the inflammatory pathway, a key component of the radiation-trauma interaction.

## **2.2 GRANULOCYTES**

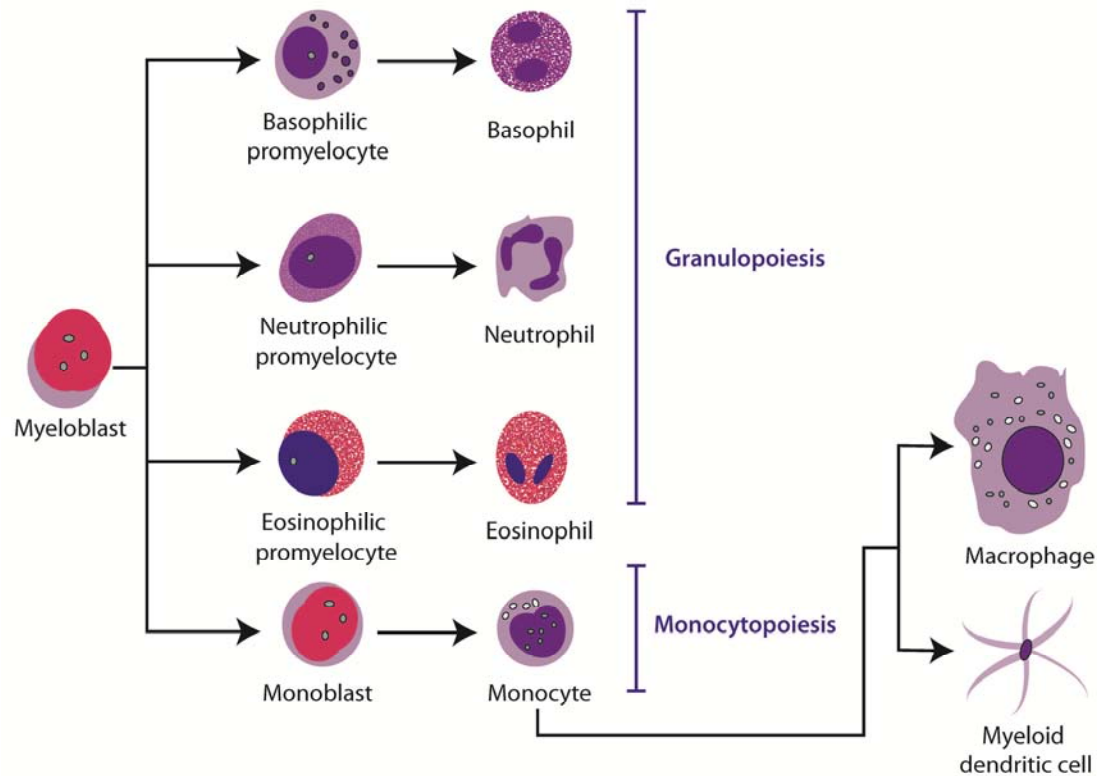
### **2.2.1 Granulocyte Functions**

Granulocytes are a category of white blood cells characterized by the presence of granules in their cytoplasm. They are also called polymorphonuclear leukocytes (PMN) because of the varying shapes of the nucleus. They contribute to the immune response by killing and phagocytizing pathogens. Neutrophils are the most abundant of the granulocytes, constituting 50% to 60% of the total circulating white blood cells. Neutrophils attack microorganisms through neutrophil extracellular traps (NETs) and the release of a variety of anti-microbial

compounds including defensins, proteolytic enzymes, myeloperoxidase, and oxygen free radicals. They remove the pathogen from the circulation by phagocytosis. Circulating neutrophils have a short lifespan, homing to marrow, spleen, or peripheral tissues and dying by apoptosis within a day (Fox *et al.* 2010, Milot and Filep 2011). In the presence of an appropriate signal, neutrophils are activated and quickly migrate from the blood to injured or infected tissue. Once the neutrophils have performed their function, they undergo apoptosis within the tissue compartment without returning to the blood (Milot and Filep 2011). In addition, there is a marginated pool of neutrophils that are sequestered within the capillary beds. These cells can be released back into the circulation.

### **2.2.2 Granulopoiesis**

Myeloblasts, the first granulocyte-specific precursors derived from the common myeloid progenitor, give rise to a series of dividing cells that include promyelocytes and myelocytes. At subsequent stages of development (metamyelocytes, band cells) the cells no longer divide but continue to differentiate and mature. Once mature, granulocytes can be released into the circulation or remain in the bone marrow. When released, the life span of granulocytes is short, only about 6.5 hours (Furze and Rankin 2008). Cytokines, growth factors, and other factors in the microenvironment modulate the processes of reproduction, maturation, and mobilization of granulocytes. Circulating granulocytes also modulate the mobilization rate. Myeloblasts also differentiate into monoblasts and from there into monocytes, which form macrophages and myeloid dendritic cells. These are the cells responsible for consuming bacteria and presenting antigenic fragments from pathogens to T lymphocytes thus activating them. Figure 3 shows the complexity of the granulopoiesis process.



**Figure 3. Granulocytic component plus macrophage**

### 2.2.3 Radiation Effects

The hematopoietic progenitor cells are very sensitive to the effects of ionizing radiation. With the death of these cells, the numbers of granulocytes in the circulation declines over the course of several weeks. Recovery occurs as the progenitor cell population becomes reconstituted and capable of generating sufficient numbers of functional cells. At moderate doses of radiation, the neutrophil count spikes within the first few hours post exposure followed by a profound neutropenia (loss of neutrophils) with a nadir at 2 to 3 weeks. The cause of the brief rise in circulating neutrophils is not known but could result from a transient increase in the release of mature cells from bone marrow or from demargination of the peripheral granulocytes (Dainiak and Waselenko 2004). Radiation casualties are commonly monitored for changes in circulating neutrophils. The existing countermeasure approved for treatment of the hematopoietic effects of radiation injury (filgrastim, the cytokine G-CSF) stimulates production of neutrophils and thereby prevents or limits this neutropenia. Stem cell transplants are also used for treatment of radiation injury (Jarrett *et al.* 2007).

### 2.2.4 Trauma Effects

Burns increase the number of bone marrow progenitor cells (Eurenius and Brouse 1973, Gamelli *et al.* 1985, Gruber and Farese 1989, Huang *et al.* 1988) and circulating granulocytes (Eurenius and Brouse 1973, Gamelli *et al.* 1985, Gruber and Farese 1989, Asko-Seljavaara 1987, El-Sonbaty and El-Otiefy 1996, Peterson *et al.* 1983) but decrease the total number of peripheral

granulocytes (Eurenus and Brouse 1973). Mobilization of mature granulocytes from bone to circulation (Rosinski *et al.* 2004, Asko-Seljavaara 1987) and production of mature granulocytes from progenitor cells in bone marrow (Rosinski *et al.* 2004) both increase. Demargination of peripheral granulocytes (Asko-Seljavaara 1987, Eurenus and Brouse 1973, Gruber and Farese 1989) occurs. In addition there seems to be a decrease in granulocyte apoptosis during the first few days after the injury (Hu and Sayeed 2004) and decreased functionality of granulocytes (Arturson 1985).

Burn with infection, however, is different from burn alone. Under these conditions there is generally a decrease in progenitor cells rather than increase (Asko-Seljavaara 1985) and a decrease in circulating granulocytes (Shoup *et al.* 1998). The myeloblast, the progenitor of both granulocytes and monocytes, is particularly affected, leading to a reduced production of granulocytes and macrophages. Endotoxin furthers this detrimental effect. The levels of G-CSF are reduced and the macrophage appears to be the major source of bone marrow suppression of granulocyte production (Gamelli *et al.* 1995).

Shock wave exposure causes rapid recruitment of granulocytes to the peripheral blood. Increased polymorphonuclear neutrophils (PMN) are seen within the first hour and this increase persists for three hours or more. A significant relative decrease of this effect is seen at six, 12, and 24 hours, which is most likely due to tissue sequestration of granulocytes, as their circulating numbers are still well above control values (Gorbunov *et al.*, 2008). There is also demargination and the acute release of granulocytes from the bone marrow, processes which are stimulated by cytokines, nitric oxide, and other materials. Vascular permeability is increased to allow for the diapedesis (infiltration) of granulocytes and macrophages into the injury site.

This phenomenon (diapedesis and tissue infiltration and sequestration) of granulocytes can be accompanied by pro-inflammatory alterations in the vascular endothelium. Trauma-induced alterations include excessive production of cytokines (several interleukins and tumor necrosis factor alpha) which can contribute to systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) (Flohé *et al.* 2003).

The normal response post wounding includes infiltration of neutrophils and macrophages into the site of the wound with eventual re-epithelialization and closure (if the wound area is not too large). With radiation, however, there is a dose-dependent decrease in infiltration and delay in re-epithelialization. Granulation tissue formation and maturation is perturbed. There is therefore a localized as well as systemic (decreased leukocytes) effect of radiation interfering with wound healing (Ran *et al.* 2004).

### **2.2.5 Role in RIPD and CI Models**

Although depletion of neutrophils (granulocytes) is one of the primary hematopoietic effects that lead to lethality, RIPD does not assess the changes in this cell population. Instead the MarCell model calculates hematopoietic death from the radiation sensitivity of an undefined, radiation-sensitive cell. New versions of a radiation and a combined injury model are likely to incorporate this endpoint since it is used clinically to monitor the health status of radiation-exposed patients.

A model of radiation effects on granulocytes has been developed by Smirnova (2010) and her group. In this model granulocytes are divided into four subpopulations: the precursor cells capable of dividing ( $X_1$ ), the non-dividing metamyelocytes and mature bone marrow granulocytes ( $X_2$ ), and granulocytes in the circulation ( $X_3$ ) and granulocytes in the body's tissues ( $X_4$ ). Chalone production from each of these populations provides feedback for the reproduction rate of the dividing cells. A limitation of this model for an integrated, physiologically-based model of radiation injury is the lack of feedback from circulating cytokines such as G-CSF, which are also used as countermeasures. Other models of hematopoiesis incorporate G-CSF feedback (see for example Obeyesekere *et al.* 2004) but these models would need to be adapted for radiation exposure. Because burn and other traumas can affect the marginated pool of granulocytes (Asko-Seljavaara 1987, Eurenus and Brouse 1973, Gruber and Farese 1989), models of combined injury will need to include this fifth subpopulation.

Granulocytes are also important for the prediction of inflammation and infection, a key intersection of radiation with traumatic injury or burn in a combined injury model. Activation of granulocytes by traumatic injury or infection results in the release of cytokines that contribute to the inflammatory response with potentially lethal consequences (Ward and Lentsch 1999, Abraham 2005, Gorbunov *et al.* 2008). Furthermore, since neutropenia is a primary target for many of the radiation countermeasures in development (Pellmar 2008), inclusion of a model of the granulocyte population provides opportunities for therapeutic inputs.

## **2.3 THROMBOCYTES**

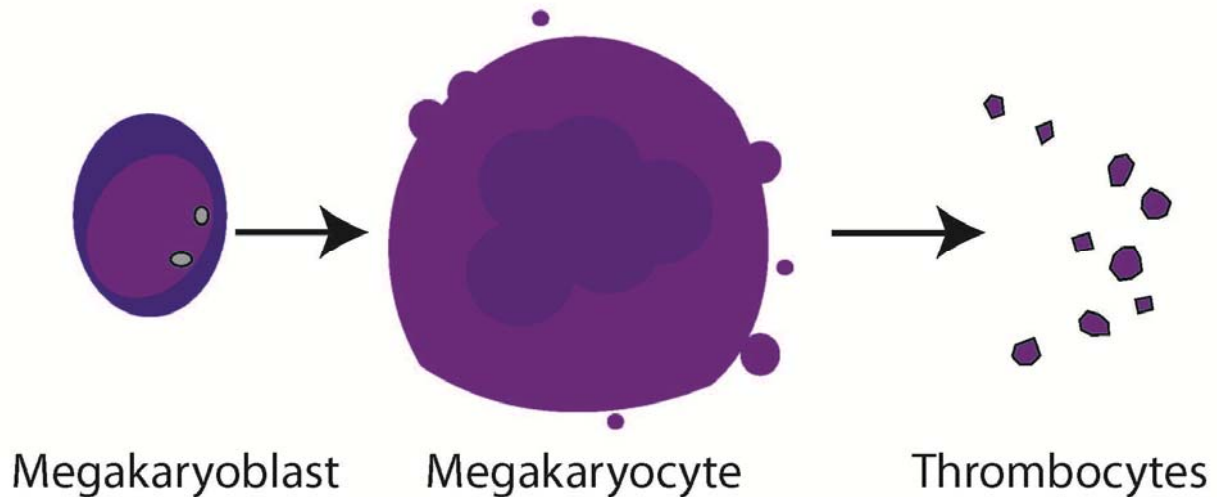
### **2.3.1 Thrombocyte Functions**

Platelets, also known as thrombocytes, play a primary role in the coagulation of blood. If the number of platelets is too low, excessive bleeding can occur; if the number of platelets is too high, blood clots can form potentially obstructing blood vessels (thrombosis). Platelets are the source of many growth factors including platelet-derived growth factor (PDGF) which regulates cell growth and division and plays a significant role in blood vessel formation (angiogenesis). Platelets also release other factors that contribute to the repair and regeneration of connective tissues and promotion of wound healing. In addition, platelets are rapidly deployed to sites of injury or infection, and potentially modulate inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines, and other inflammatory mediators.

### **2.3.2 Thrombopoiesis**

The thrombopoiesis process is shown graphically in Figure 4; thrombocytes are derived from the common myeloid progenitor cell, the megakaryoblast. When a megakaryoblast begins to reproduce its nuclear chromatin without undergoing mitosis, a promegakaryocyte is formed. The promegakaryocyte does not divide but grows in size and differentiates into a megakaryocyte. A megakaryocyte is extremely large in size, with a multi-lobed nucleus containing up to 256 times the normal chromosome complement and exhibiting long cytoplasmic extensions. Platelets are formed by the pinching off these extensions and nuclear material. One

megakaryocyte can give rise to 3000-4000 platelets. The processes of reproduction and differentiation are controlled by a variety of factors in the microenvironment, including thrombopoietin (Kaushansky *et al.* 2009). Once released into the circulation, the lifespan of a platelet averages 10 days.



**Figure 4. Thrombopoietic component**

### **2.3.3 Radiation Effects**

Like neutropenia, thrombocytopenia is a consequence of lethal effects of radiation on the hematopoietic progenitor cells in the bone marrow. Loss of myeloid progenitor cells leads to a decline in the circulating thrombocytes through attrition. After moderate doses of radiation, platelets in circulation are sustained at relatively normal levels for several days, but then decline and reach a nadir between 2 to 3 weeks after exposure. Platelet transplants are often necessary to promote survival after lethal doses.

Like neutrophils, platelet levels provide a clinical endpoint that is frequently monitored after radiation injury to assess the health status of the patient. Some investigators (Stickney *et al.* 2005, Moroni *et al.* 2011) have noted that the duration of the thrombocytopenia after radiation exposure is more predictive of hematopoietic lethality than neutropenia. In our prototype model, a logit analysis indicated that thrombocytopenia was a stronger predictor of lethality than granulocytopenia, in agreement with this perspective.

### **2.3.4 Trauma Effects**

Within an hour of a burn injury (El-Sonbaty and El-Otiefy 1996, Eurenus *et al.* 1972, Fujimi *et al.* 2006, Kalmaz and Guest 1991) the number of circulating platelets shows a transient decrease. Mild thrombocytosis occurs between day 6 and day 30. Burns alter thrombopoiesis (Kalmaz and Guest 1991) with an immediate decrease in lifespan of platelets that returns to normal at day 30.

Platelet yield also decreases by 60% during the first 5 days. There is an abrupt increase in megakaryocytes in the bone marrow. Turnover time for platelets is 2-3 times normal during the first 30 days after burn injury.

There are three areas of tissue damaged by burn: a central area of coagulation, an outer ring of hyperemia, and an area of stasis in between. While circulation is intact in the hyperemic zone, the intermediate zone develops dilated microvasculature with endothelial leakage of plasma and intravascular proteins. The coagulation cascade is initiated and the inflammatory response is aggravated in this region by the release of prostaglandins, histamine, bradykinin and possibly other chemical mediators. Interstitial edema decreases blood flow, causing this to become the zone of stasis. Progressive vascular occlusion occurs and, if not treated, the zone of stasis can become a full-thickness injury (Edlich 2010).

In hemorrhage the clotting process begins at the site of injury of the endothelium. Collagen, along with several clotting factors, is released and binds circulating platelets to the site of injury. Activation of platelets by this adhesion process causes them to release several factors, which in turn activate other platelets to adhere and form a plug. (This is the cellular component of the coagulation process; several proteins and other factors are also involved in a complex cascade of events, which will not be described in detail here.) Some products of the coagulation system can also increase vascular permeability as well. In major hemorrhage the number of platelets may be insufficient to control the hemorrhage, in which case hypoperfusion, hypovolemic shock, and death can result. If the hemorrhage is controlled, the repair process begins, the clots are slowly resolved, and the platelets are phagocytized.

In severe hemorrhage the prothrombin (PT) or partial thromboplastin time (PTT) may increase, which indicates coagulopathy involving the non-cellular component of the clotting cascade. These are independent predictors of all-cause mortality.

### **2.3.5 Role in RIPD and CI Models**

Thrombopoiesis is not currently incorporated into the RIPD model. Smirnova (2010) and her colleagues have developed a model of thrombopoiesis that considers three compartments: the progenitor cells in the bone marrow ( $X_1$  cells), the non-dividing cells of the bone marrow ( $X_2$  cells) and the thrombocytes in the circulation ( $X_3$  cells). These populations provide feedback on the reproductive rate of the  $X_1$  cells through chalone production.

Platelets, in combination with leukocytes, contribute to inflammatory processes (Gawaz *et al.* 2005, Rodrigues and Granger 2010). With vascular injury (i.e., when the endothelial layer is disrupted), platelets adhere to the vascular endothelium, become stimulated, and release pro-inflammatory mediators. Adherent platelets attract leukocytes to the site and thereby increase the inflammatory processes. Release of a variety of mediators with inflammation results in a widening of the space between the endothelial cells of the vascular wall allowing fluids and solutes to pass, and thereby increases vascular permeability (Rodrigues and Granger 2010). Platelets play a role in maintaining the integrity of the vascular wall (Nachman and Rafii 2008). With thrombocytopenia, vascular permeability increases allowing local movement of erythrocytes into the tissue which causes petechiae, a common symptom of radiation exposure.

Since inflammation is a key area of interaction of radiation and trauma, the impact of platelets through these pathways may be an important consideration in the modeling of combined injury.

## 2.4 ERYTHROCYTES

### 2.4.1 Erythrocyte Functions

Erythrocytes, or red blood cells, are responsible for transporting oxygen from the lungs to the tissues and returning carbon dioxide. Transport of oxygen is greatly enhanced by hemoglobin molecules contained in erythrocytes; although oxygen can dissolve directly into the pulmonary capillaries, the total amount dissolved would be far below the quantities needed to sustain tissue function. Almost all of the erythrocyte dry mass is made up of hemoglobin, which transports the carbon dioxide produced in cellular respiration back to the lungs for exhalation (though 90% of carbon dioxide produced by tissue respiration is dissolved in the blood rather than bound to the globin protein). Release of oxygen from the hemoglobin into the tissue cells is promoted by the low pH, high CO<sub>2</sub> content, and high concentration of 2, 3-bisphosphoglyceric acid found in the tissues. Hemoglobin also transports nitric oxide, which is important in cellular signaling processes, and releases it in tissues.

### 2.4.2 Erythropoiesis

As shown in Figure 5, proerythroblasts are the first identifiable precursors of erythrocytes in the bone marrow. These cells reproduce and differentiate into a series of mitotic cells that include the basophilic erythroblasts and the early and middle polychromatophil normoblasts. At the stage of the late polychromatophil normoblast, cell division ceases. Differentiation continues with the formation of the reticulocyte and erythrocyte stages. Mature erythrocytes exit the bone marrow into the circulating peripheral blood. Erythropoietin and numerous other factors modulate the processes of erythropoiesis.

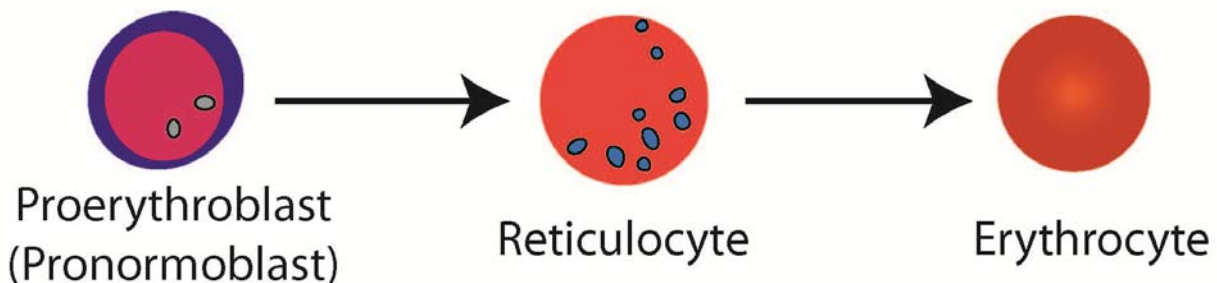


Figure 5. Erythrocytic component

### 2.4.3 Radiation Effects

Mature erythrocytes are less radiation sensitive than the other blood elements, and the pattern of depletion and recovery differs in that the nadir is not as pronounced and occurs much later than for lymphocytes, granulocytes, and platelets (in that order). This happens despite the radiation sensitivity of erythroblasts (Davis 1954). However, radiation exposure does impair the oxygen-carrying capacity of the blood (Puchala *et al.* 1999), and transfusion requirements of both platelets and red cells at Chernobyl were generally higher than expected (Gusev *et al.* 2001).

### 2.4.4 Trauma Effects

In the normal steady state a small number of hematopoietic progenitor cells (HPC) are released from the bone marrow into the peripheral circulation and then home back to the bone marrow so that circulating cells are available to fill open bone marrow niches. This is a homing effect, not random deposition (Wright *et al.* 2001). In trauma and hemorrhagic shock, bone marrow production of HPCs is suppressed and the HPCs are released and migrate to the site(s) of tissue trauma and other tissue sites to a lesser extent. Hemorrhagic shock exacerbates this migration (Badami *et al.* 2007). Besides erythroid colony-forming units in the bone marrow, granulocyte-macrophage colony-forming units are suppressed as well until recovery begins and HPC growth in the peripheral tissues returns toward baseline. This may be related to a hypercatecholamine state induced by trauma. At normal physiological levels catecholamines increase erythropoiesis, but at the very high levels seen post traumatic injury they depress it (Fonseca *et al.* 2005). Peripheral reticulocyte counts are low despite adequate iron stores and erythropoietin levels, indicating that trauma depresses erythropoiesis in the bone marrow by mechanisms not completely understood (Livingston *et al.* 2003). (It should be noted that one study (Ran *et al.* 2007) demonstrated that addition of serum from a rat model with burn or combined radiation-burn injuries significantly elevated the number of HPCs; serum from irradiated only rats inhibited HPC growth. This may be related to the different levels of TNF-alpha and IL-6 production in the different groups.)

In trauma hemorrhage, if significant enough, reduces the availability of oxygen to the tissues. The resulting tissue hypoxia causes a buildup of lactic acid, which can lead to acidosis and cell death. Local hypoxia can cause vasodilation, which if the integrity of the vasculature has been compromised by trauma can lead to more blood loss (except in the lungs, where hypoxia induces vasoconstriction). Dead erythrocytes within the body are broken down by phagocytic leukocytes and products (iron, carbon monoxide, and bilirubin) are released by granulocytes, particularly neutrophils. The released bilirubin can, if accumulated too rapidly, clog capillaries and terminal arterioles. Substantial blood loss can occur in burns as well, and excision of large burn wounds (preferably early post injury) may be required to stabilize the patient.

### 2.4.5 Role in RIPD and CI Models

Erythrocyte damage or loss is not a factor in the current RIPD model. Smirnova (2010) has modeled erythropoiesis in a manner similar to other blood cells. Three compartments (dividing cells of the bone marrow, non-dividing cells of the bone marrow, and circulating cells) are considered. Because changes in erythrocytes can impact metabolic activity, which is seriously

compromised after burn injury for example, it may important for future iterations of the combined injury model to incorporate the process of erythropoiesis.

### **3.0 SUMMARY**

Changes in hematopoiesis are evident with many different kinds of injury, including radiation, burn, and mechanical trauma. This report reviews the consequences of these various insults on the changes of circulating lymphocytes, granulocytes, thrombocytes, and erythrocytes and the cellular processes involved. The effects of any one insult are often complex, including altering many of the hematopoietic components. The complexity is increased in the context of combined injuries because the responses of the hematopoietic system to injury often differ with respect to the type of injury. The responses of the individual hematopoietic components to different insults vary not only in magnitude but sometimes in direction as well.

Hematopoietic effects are an important part of describing and predicting the acute morbidity and mortality resulting from injury. Physiologically-based models will need to incorporate the effects of the various insults, alone and in combination. To aid in the process of incorporating the hematopoietic effects into the models, this report reviews the major pathways of hematopoiesis. The modulation of these pathways by the insult(s) is integral to understanding and mathematically describing the effects of the insults. As the models mature, it is likely that the hematopoietic system will be represented greater with increasing sophistication in order to better define this critical component of injury.

## 4.0 REFERENCES

- Abraham E. Alterations in cell signaling in sepsis. Clinical Infectious Diseases 2005 Nov; **41**:S459-S464.
- Arturson G. Neutrophil granulocyte functions in severely burned patients. Burns Incl Therm Inj 1985 Jun; **11**(5):309-319.
- Asko-Seljavaara S. Altered cell proliferation in burns. J Trauma 1985 Feb; **25**(2):101-105.
- Asko-Seljavaara S. Granulocyte kinetics in burns. J Burn Care Rehabil 1987 Dec; **8**(6):492-495.
- Badami C.D., D.H. Livingston, Z.C. Sifri, F.J. Caputo, L. Bonilla, L.M. Mohr, E.A. Deitch (2007). Hematopoietic Progenitor Cells Mobilize to the Site of Injury After Trauma and Hemorrhagic Shock in Rats. Journal of Trauma® Injury, Infection, and Critical Care **63**(6):596-602.
- Barlow Y. T lymphocytes and immunosuppression in the burned patient: a review. Burns 1994 Dec; **20**(6):487-490.
- Bower J.E., P.A. Ganz, N. Aziz, J.L. Fahey, S.W. Cole. T-cell homeostasis in breast cancer survivors with persistent fatigue. JNCI Journal of the National Cancer Institute 2003; **95**(15):1165-1168.
- Bower J.E., P.A. Ganz, M.L. Tao, W. Hu, T.R. Belin, S. Sepah, S. Cole, N. Aziz. Inflammatory biomarkers and fatigue during radiation therapy for breast and prostate cancer. Clin Cancer Res 2009 Sep; **15**(17):5534-5540.
- Burleson D.G., A.D. Mason, B.A. Pruitt. Lymphoid subpopulation changes after thermal injury and thermal injury with infection in an experimental model. Ann Surg 1988 Feb; **207**(2):208-212.
- Chaplin D.D. Overview of the immune response. J Allergy Clin Immunol. 2010 Feb; **125**( Suppl 2):S3-23.
- Dainiak N, J.K. Waselenko. Biology and clinical features of radiation injury in adults [Internet]. UpToDate® 2004. Available from:  
<http://www.sassit.co.za/Journals/General%20complications/UpToDate%C2%AE%20%27Biolog%20and%20clinical%20features%20of%20radiation%20injury.htm>
- Diacovo T.G., K.D. Puri, R.A. Warnock, T.A. Springer, U.H. von Andrian. Platelet-mediated lymphocyte delivery to high endothelial venules. Science 1996 Jul; **273**(5272):252-255.

Ditschkowski M., E. Kreuzfelder, V. Rebmann, S. Ferencik, M. Majetschak, E.N. Schmid, U. Obertacke, H. Hirche, U.F. Schade, H. Grosse-Wilde. HLA-DR expression and soluble HLA-DR levels in septic patients after trauma. Ann Surg 1999 Feb; **229**(2):246-254.

El-Sonbaty M.A., M.A. El-Otiefy. Haematological change in severely burned patients. Annals of Burns and Fire Disasters 1996; **IX**(4). Accessed on line at:  
[http://www.medbc.com/annals/review/vol\\_9/num\\_4/text/vol9n4p206.htm](http://www.medbc.com/annals/review/vol_9/num_4/text/vol9n4p206.htm)

Edlich R. Thermal Burns [Internet]. Medscape Reference 2010 Mar; Available from:  
<http://emedicine.medscape.com/article/1278244-overview>

Eurenius K., R.O. Brouse. Granulocyte kinetics after thermal injury. Am J Clin Pathol 1973 Sep; **60**(3):337-342.

Eurenius K., R.F. Mortensen, P.M. Meserol, P.W. Curreri. Platelet and megakaryocyte kinetics following thermal injury. J Lab Clin Med 1972 Feb; **79**(2):247-257.

Fayazov A.D., S.I. Shukurov, B.I. Shukurov, B.C. Sultanov, A.N. Namazov, D.A. Ruzimuratov. Disorders of the immune system in severely burned patients. Ann Burns Fire Disasters 2009 Sep; **22**(3):121-130.

Flohé S., S. Lendemans, C. Selbach, C. Waydhas, M. Ackermann, F.U. Schade, E. Kreuzfelder. Effect of granulocyte-macrophage colony-stimulating factor on the immune response of circulating monocytes after severe trauma. Crit Care Med 2003 Oct; **31**(10):2462-2469.

Fonseca R.B., A.M. Mohr, L. Wang, Z.C. Sifri, P. Rameshwar, D.H. Livingston. The impact of a hypercatecholamine state on erythropoiesis following severe injury and the role of IL-6. J Trauma 2005 Oct; **59**(4):884-889.

Fox S., A.E. Leitch, R. Duffin, C. Haslett, A.G. Rossi. Neutrophil apoptosis: relevance to the innate immune response and inflammatory disease. J Innate Immun. 2010; 2(3):216-227.

Fujimi S., M.P. MacConmara, A.A. Maung, Y. Zang, J.A. Mannick, J.A. Lederer, P.A. Lapchak. Platelet depletion in mice increases mortality after thermal injury. Blood 2006 Jun; **107**(11):4399-4406.

Furze R.C., S.M. Rankin. Neutrophil mobilization and clearance in the bone marrow. Immunology 2008 Nov; **125**(3):281-288.

Gamelli R.L., J.C. Hebert, R.S. Foster Jr. Effect of burn injury on granulocyte and macrophage production. J Trauma 1985 Jul; **25**(7):615-619.

Gawaz M., H. Langer, A.E. May. Platelets in inflammation and atherogenesis. J Clin Invest 2005 Dec; **115**(12):3378-3384.

Gorbunov N.V., L.V. Asher, N.M. Elsayed, J.L. Atkins. Inflammatory Response in Primary Blast Injury. In N.M. Elsayed and J.L. Atkins (eds), Explosion and Blast-Related Injuries. Elsevier Academic Press, Burlington, MA. 2008.

Gruber D.F., A.M. Farese. Bone marrow myelopoiesis in rats after 10%, 20%, or 30% thermal injury. J Burn Care Rehabil 1989 Oct; **10**(5):410-417.

Gusev I.A., A.K. Guskova, F.A. Mettler Jr. (eds). Medical Management of Radiation Accidents (2<sup>nd</sup> edition). CRC Press, Boca Raton, FL. 2001.

Hu Z., M.M. Sayeed. Suppression of mitochondria-dependent neutrophil apoptosis with thermal injury. American Journal of Physiology - Cell Physiology 2004 Jan; **286**(1):C170 -C178.

Huang W.H., J.Z. Wu, Z.X. Hu, S.Z. Feng, X.Y. Huang, A. Li, W.A. Robinson. Bone marrow granulopoietic response to scalds and wound infection in mice. Burns Incl Therm Inj 1988 Aug; **14**(4):292-296.

Jarrett D.G., R.G. Sedlak, W.E. Dickerson, G.I. Reeves. Medical treatment of radiation injuries—Current US status. Radiat Meas 2007; **42**:1063-1074.

Jarvis W.R., Bennett and Brachman's Hospital Infections (5<sup>th</sup> edition). Wolters Kluwer/Lippincott Williams and Wilkins, Philadelphia. 2007.

Jones T.D., M.D. Morris, R.W. Young. Dose-rate RBE factors for photons: hematopoietic syndrome in humans vs stromal cell cytopenia. Health Physics 1994; **67**(5):495-508.

Kalmaz G.D., M.M. Guest. Burn trauma: role of thrombocytopenia in inhibition of megakaryocytopoiesis. Ann NY Acad Sci 1991; **628**:396-398.

Lannacone M., G. Sitia, M. Isogawa, P. Marchese, M.G. Castro, P.G. Lowenstein, F.V. Chisari, Z.M. Ruggeri, L.G. Guidotti. Platelets mediate cytotoxic T lymphocyte-induced liver damage. Nat Med 2005 Nov; **11**(11):1167-1169.

Lederer J.A., B.H. Brownstein, M.D. Lopez, S. MacMillan, A.J. Delisle, M.P. MacConmara, M.A. Choudhry, W. Xiao, S Lekousi, J.P. Cobb, H.V. Baker, J.A. Mannick, I.A. Chaudry and others. Comparison of longitudinal leukocyte gene expression after burn injury or trauma-hemorrhage in mice. Physiological Genomics 2008; **32**:299-310.

Livingston D.H., D. Anjaria, J. Wu, C.J. Hauser, V. Chang, E.A. Deitch, P. Rameshwar. Bone marrow failure following severe injury in humans. Ann Surg 2003 Nov; **238**(5):748-753.

Lorusso L., S.V. Mikhaylova, E. Capelli, D. Ferrari, G.K. Ngonga, G. Ricevuti. Immunological aspects of chronic fatigue syndrome. Autoimmun Rev 2009 Feb; **8**(4):287-291.

MacLeod J.B., M Lynn, M.G. McKenney, S.M. Cohn, M. Murtha. Early coagulopathy predicts mortality in trauma. J Trauma 2003 Jul; **55**(1):39-44.

- Milot E. and J.G. Filep. Regulation of neutrophil survival/apoptosis by Mcl-1. Scientific World Journal 2011 Oct; **11**:1948-1962.
- Moroni M., E. Lombardini, R. Salber, M. Kazemzadeh, V. Nagy, C. Olsen, M.H. Whitnall. Hematological changes as prognostic indicators of survival: similarities between Gottingen minipigs, humans, and other large animal models. PLoS One. 2011; **6**(9):e25210. Epub 2011 Sep 28.
- Nachman R.L., S. Rafii. Platelets, petechiae, and preservation of the vascular wall. N Engl J Med 2008 Sep; **359**(12):1261-1270.
- Nagayama H., J. Ooi, A. Tomonari, T. Iseki, A. Tojo, K. Tani, T.A. Takahashi, N. Yamashita, A. Shigetaka. Severe immune dysfunction after lethal neutron irradiation in a JCO nuclear facility accident victim. Int J Hematol 2002 Aug; **76**(2):157-164.
- Nikolajczyk B.S. B cells as under-appreciated mediators of non-auto-immune inflammatory disease. Cytokine 2010 Jun; **50**(3):234-242.
- Obeyesekere M.N, B.B. Berry, P.P. Spicer, M. Korbling. A mathematical model of haemopoiesis as exemplified by CD34+ cell mobilization into the peripheral blood. Cell Prolif **37**(4), 279-294.
- Parreira J.G., S. Rasslan, L.F. Poli de Figueiredo, T.C. Bortolheiro, S. Sinosaki, D. Hardt, M.Y. Langui, M.N. Rocha, C.A. Longui, C. Chiattoni, M. Rocha e Silva. Impact of shock and fluid resuscitation on the morphology and apoptosis of bone marrow: an experimental study. J Trauma 2004 May; **56**(5):1001-1007.
- Pellmar T.C. and D. Oldson. FW Module: Overview of the Fatigability and Weakness Module in RIPD. ARA Technical Note ARA/HS-TN-11-010-A. 2011.
- Pellmar T.C. Bone Marrow Niches in Hematopoiesis. ARA Technical Note ARA/HS-TN-11-009-A. 2011.
- Pellmar T.C. Development of radiation countermeasures. In: Wiley Handbook of Science and Technology for Homeland Security. Hoboken, NJ, USA: John Wiley and Sons, Inc.; 2008. Available from: <http://doi.wiley.com/10.1002/9780470087923.hhs168>
- Peterson V., J. Hansbrough, C. Buerk, C. Rundus, S. Wallner, H. Smith, W.A. Robinson. Regulation of granulopoiesis following severe thermal injury. J Trauma 1983 Jan; **23**(1):19-24.
- Puchała M., Z. Szewda-Lewandowska. Damage to hemoglobin by radiation-generated serum albumin radicals. Free Radic Biol Med 1999 May; **26**(9-10):1284-1291.
- Qu Y., S. Jin, A. Zhang, B. Zhang, X. Shi, J. Wang, Y. Zhao. Gamma-ray resistance of regulatory CD4+ CD25+ Foxp3+ T cells in mice. Radiation Research 2010; **173**(2):148-157.

Ran X., T. Cheng, C. Shi, H. Xu, J. Qu, G. Yan, Y. Su, W. Wang, R. Xu. The effects of total-body irradiation on the survival and skin wound healing of rats with combined radiation-wound injury. J Trauma 2004 Nov; **57**(5):1087-1093.

Ran X.Z., Y.P. Su, Z.W. Zong, C.H. Guo, H.E. Zheng, X.H. Chen, G.P. Ai, T.M. Cheng. Effects of serum from rats with combined radiation-burn injury on the growth of hematopoietic progenitor cells. J Trauma. 2007 Jan; **62**(1):193-198.

Rodrigues S.F., D.N. Granger. Role of blood cells in ischaemia-reperfusion induced endothelial barrier failure. Cardiovasc Res 2010 Jul; **87**(2):291-299.

Rosinski M., M.L. Yarmush, F. Berthiaume. Quantitative dynamics of in vivo bone marrow neutrophil production and egress in response to injury and infection. Ann Biomed Eng 2004 Aug; **32**(8):1108-1119.

Schlüter B., W. König, M. Köller, G. Erbs, F.E. Müller. Studies on B-lymphocyte dysfunctions in severely burned patients. J Trauma 1990 Nov; **30**(11):1380-1389.

Schwacha M.G. Gammadelta T-cells: potential regulators of the post-burn inflammatory response. Burns 2009 May; **35**(3):318-326.

Shoup M., J.M. Weisenberger, J.L. Wang, J.M. Pyle, R.L. Gamelli, R. Shankar. Mechanisms of neutropenia involving myeloid maturation arrest in burn sepsis. Ann Surg 1998 Jul; **228**(1):112-122.

Stedman, T. Stedman's Medical Dictionary. 3rd edition. Baltimore: Lippincott Williams and Wilkins; 2006.

Stickney D.R., C. Dowding, S. Authier, A. Garsd, N. Onizuka-Handa, C. Reading, J.M. Frincke. 5-androstenediol improves survival in clinically unsupported rhesus monkeys with radiation-induced myelosuppression. Int Immunopharmacol 2007 Apr; **7**(4):500-505.

Sugarman S.L., R.E. Goans, A.S. Garrett, G.K. Livingston. The Medical Aspects of Radiation Incidents. Oak Ridge Associated Universities under contract from Department of Energy; Oak Ridge, TN. 2010.

Wagner D.D., P.C. Burger. Platelets in inflammation and thrombosis. Arterioscler Thromb Vasc Biol 2003 Dec; **23**(12):2131-2137.

Ward P.A., A.B. Lentsch. The acute inflammatory response and its regulation. Arch Surg 1999 Jun; **134**(6):666-669.

Weyrich A.S., G.A. Zimmerman. Platelets: signaling cells in the immune continuum. Trends Immunol 2004 Sep; **25**(9):489-495.

Wright D.E., A.J. Wagers, A.P. Gulati, F.L. Johnson, I.L. Weissman. Physiological migration of hematopoietic stem and progenitor cells. Science 2001 Nov 30; **294**(5548):1933-1936.

Zukhbaya T.M., O.A. Smirnova. An experimental and mathematical analysis of lymphopoiesis dynamics under continuous irradiation. Health Phys 1991 Jul; **61**(1):87-95.

## **APPENDIX A. ACRONYMS**

**CI** – Combined Injury

**HPC** - Hematopoietic Progenitor Cells

**MODS** - Multiple Organ Dysfunction Syndrome

**NET** - Neutrophil Extracellular Traps

**PDGF** - Platelet-Derived Growth Factor

**PMN** - Polymorphonuclear Leukocytes

**PT** – Prothrombin

**PTT** - Partial Thromboplastin Time

**RIPD** - Radiation-Induced Performance Decrement

**SIRS** - Systemic Inflammatory Response Syndrome

THIS PAGE IS INTENTIONALLY LEFT BLANK.

## APPENDIX B. GLOSSARY

**Apoptosis**—programmed cell death, causing cells to fragment into membrane-bound particles that are phagocytized by other cells. It can be a natural process (elimination of transitory organs and tissues during the embryonic stage or after about 50 cell divisions) or triggered by injury, infection, or circulatory impairment. Cytokines can either precipitate or suppress apoptosis.

**Chemokines**—small cytokines that stimulate leukocyte movement and attraction. This movement is called chemotaxis.

**Colony stimulating factor (CSF)**—glycoproteins that bind to receptor proteins on the surface of hematopoietic stem cells, causing them to proliferate and differentiate into a specific kind of blood cells. Two CSFs used in stimulating irradiated bone marrow to regenerate are granulocyte (G-CSF) and granulocyte and macrophage (GM-CSF) colony stimulating factors.

**Cytokines**—small proteins secreted by various cells that regulate immune responses by modulating the maturation, growth, and responsiveness of particular cell populations. They sometimes enhance or inhibit the action of other cytokines. Interactions among cytokines during the immune response can be extremely complex.

**Dendritic cells**—monocytes present in tissues in contact with the external environment (skin, mucous membranes of digestive and respiratory tracts). When they encounter pathogens they become activated, phagocytize portions of the bacteria, virus, or other foreign material, and migrate to lymph nodes to present the protein fragments to T lymphocytes, thus activating them in turn.

**Disseminated intravascular coagulation (DIC)**—a systemic complication of an underlying disorder resulting in activation of a systemic inflammatory response, intravascular coagulation, depleting of clotting factors, and end-organ damage. DIC is often associated with or precipitated by trauma, especially neurotrauma, as well as toxic reactions and possibly ionizing radiation.

**Epithelium**—cellular layer covering all cutaneous, mucous, and serous surfaces, including the gastrointestinal tract. Is either on or opens to the outside of the body.

**Endothelium**—the layer of flat cells lining the blood and lymphatic vessels (as well as the heart).

**Erythrocytes**—red blood cells responsible for tissue respiration (supply oxygen to cells). They have a limited role in the immune response in that hemoglobin releases free radicals when the erythrocyte cell membrane is lysed, thus damaging the pathogen. They also release compounds that serve as vasodilators. The time for differentiation from stem cell to anucleated erythrocyte takes about a week and occurs in the red bone marrow. Life in the peripheral circulation is normally 100-120 days before erythrocytes become senescent, are consumed by macrophages, and their components recycled.

**Granulocytes**—leukocytes containing granules in the cytoplasm. These granules store several different cytotoxic materials as well as other substances, and are released when the granulocyte is stimulated immunologically. When granulocytes are stained by Wright’s stain, they may appear red (eosinophils), blue (basophils), or take up little or no stain (neutrophils), depending upon what materials are stored in the cell’s granules. Of these three types of granulocytes, neutrophils are the most common.

**Histiocyte**—literally, “tissue cell”. These are monocytes that migrate from the bone marrow to most tissues of the body where they mature and differentiate into tissue-specific macrophages.

**Inflammation**—the complex and dynamic process that occurs in affected blood vessels and adjacent tissues in response to injury by physical, radiological, chemical, or biological agents and creates morphologic changes. Materials that created the insult are removed, along with damaged tissue, as part of the repair process. Clinically tissues respond to inflammation with erythema (rubor), swelling (tumor), pain (dolor), and warmth (calor).

**Leukocytes**—white blood cells. They move between the lymphatic system, peripheral blood, and tissues. The three major groups are lymphocytes, monocytes, and granulocytes. They are rapidly transported to sites of inflammation and infection.

**Lymphocytes**—a class of leukocytes that is formed in the marrow from undifferentiated stem cells and then migrates to lymphatic tissue throughout the body. There are two main groups (B cells and T cells) that function in maintenance of humoral immunity and cell-mediated immunity respectively. A third form of lymphocyte, natural killer cells, is involved in the destruction of tissue cells that have been altered by infection, tumor, or other factors.

**Macrophages**—as the name (“large eater”) implies, they consume tumor cells, bacteria, and inert foreign material and present fragments of these targets as antigens to lymphocytes in the lymph nodes. They are widely distributed throughout the body. They also consume dead normal tissue cells and their debris.

**Mast cells**—are found in connective and mucosal tissues. They contain many basophilic cytoplasmic granules that secrete heparin, histamine, and other cytokines important in the immune response. They are involved in immediate hypersensitivity immune reactions. Though similar in appearance to basophils, they originate from different precursor cells in the bone marrow.

**Monocytes**—large mononuclear leukocytes that play several roles in the immune system and inflammatory response. After a few days in the blood stream they migrate into loose connective tissue and differentiate into macrophages or dendritic cells. They are involved in phagocytosis, cytokine production, and antigen presentation to activate T lymphocytes.

**Multiple organ dysfunction syndrome (MODS)**—a disorder characterized by severe systemic inflammation and progressive and sequential loss of function in several vital organs. It is associated with SIRS and can be caused by ionizing radiation, certain chemicals, severe trauma, and infectious or noninfectious processes triggering this inflammation. As severity increases and

organ(s) become dysfunctional or nonfunctional, it is sometimes called multiple organ failure (MOF).

**Phagocytosis**—ingestion and digestion of cells, bits of necrotic tissue, foreign materials, etc. by certain leukocytes. Granulocytes (primarily neutrophils) chiefly ingest bacteria, while macrophages, mononucleated cells (histiocytes and monocytes), and dendritic cells ingest dead tissue and degenerated cells.

**Polymorphonuclear leukocyte (PMN)**—common term used for granulocytes, particularly neutrophils.

**Plasma cells**—a type of B lymphocyte active in the formation and secretion of antibodies. They are formed in the bone marrow, migrate to lymph nodes, and mature there.

**Platelets**—thrombocytes; fragments of megakaryocytes that function in clotting as well as in modulation of inflammation.

**Reticulocytes**—young erythrocytes with a mesh-like network of ribosomal RNA that is visible with certain stains. Normally they appear during the last day or two of erythropoiesis in the bone marrow and the ribosomal DNA remains stainable for the first day post release into the circulation. They normally comprise about 1% of circulating erythrocytes but this percentage increases during hemorrhage. A percentage less than 1% indicates problems with erythrocyte production, often due to chemotherapy or other toxic agents.

**Systemic Inflammatory Response Syndrome (SIRS)**—a clinical response to a nonspecific insult characterized by at least two of four variables: fever  $>38^{\circ}$  or  $<36^{\circ}$ ; heart rate  $>90/\text{min}$ ; respiratory rate  $>20/\text{min}$  or a  $\text{PaCO}_2 <32 \text{ mm Hg}$ ; abnormal leukocyte count ( $>12,000/\mu\text{L}$ ;  $<4,000/\mu\text{L}$ ; or  $>10\%$  bands). Causes include trauma, infection, inflammation, and possibly radiation.

THIS PAGE IS INTENTIONALLY LEFT BLANK.

**APPENDIX C. DISTRIBUTION LIST**  
**DTRA-TR-12-16**

**DEPARTMENT OF DEFENSE**

Defense Technical Information Center  
8725 John J. Kingman Road, Suite 0944  
Fort Belvoir, VA 22060-6201  
ATTN: DTIC/OCA

Defense Threat Reduction Agency  
8725 John J. Kingman Road, RD-NTSN  
Fort Belvoir, VA 22060-6201

Defense Threat Reduction Information Analysis Center  
1680 Texas Street, S.E.  
Kirtland AFB, NM 87117

**DEPARTMENT OF DEFENSE CONTRACTORS**

ARA, Inc.  
801 N. Quincy Street, Suite 700  
Arlington, VA 22203

THIS PAGE IS INTENTIONALLY LEFT BLANK.