Chapter IX: Acute Radiation Effects: Organ Specific Organs Dose and Species Differences:

Section C: Immune System

Disturbance of Immune Homeostasis by Radiation

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A. Introduction

The immune system has evolved a panoply of mechanisms to cope with the numerous diverse challenges that continually threaten to destroy tissue function. These same immune mechanisms are invoked in response to ionizing radiation (IR).

At the highest level of distinction, immune mechanisms can be considered as belonging to either innate or adaptive immune systems. Some of the properties of these systems are presented in figure 1.

Innate Immune Mechanisms

- Evolved first with the development of multicellular organisms.
- Are pre-existing or natural first responders in defense.
- Use pattern recognition receptor (PRRs) systems to recognize “dangerous” pathogens or effete normal tissue products that they bind, engulf or surround, and neutralize.
- Phagocytes (neutrophils/macrophages) are the major effectors, but PRRs are expressed by all cell types and signal the existence of “danger”.
- They aim to maintain body integrity

Adaptive Immune Mechanisms

- Mediated by subpopulations of T or B lymphocytes with receptors that are specific for small peptide Ags, each cell expressing receptors of one specificity.
- T helper (Th) cells recognize peptide antigens processed so as to bind major histocompatibility complex (MHC) class II “self” molecules for presentation. Cytotoxic T cells recognize peptide antigens processed and presented by MHC class I “self” molecules. B cells recognize antigens independent of MHC.
- T and B cells exposed to antigen are induced to proliferate and differentiate into effector cells; the former participates in cell-mediated immunity, the latter differentiate into antibody producing cells, participating in humoral immunity.
- Immunological memory is generated in both T and B cell compartments. It is long-lasting and rapidly responds to further Ag-specific challenge.

Figure 1: Attributes of Innate and Adaptive Immune Mechanisms.

Innate immunity developed earlier in evolution, but as adaptive immunity emerged, major routes of communication developed to coordinate responses between the two systems, for example certain innate cell populations became specialized so that they could present antigen (Ag) to lymphocytes to generate Ag-specific adaptive immunity. Responses by

the two immune systems were synchronized by small molecules called cytokines and chemokines and ligand-receptor interactions working within communication networks.

B. Innate Immunity

The innate immune system is the first non-cell-intrinsic cellular defense against host invasion by infectious, and other, agents. All cells exert some level of intrinsic resistance, but mobile myeloid phagocytic cells are actively recruited to sites of "danger" and damage, where they assess the extent of the problem and make measured responses to deal with it. Although they are considered to have evolved to kill, or limit the spread of, infectious agents and, later, to repair the damage caused, they also respond to non-infectious challenges, like IR.

Inflammation: Myeloid cell mobilization and recruitment is controlled by the concerted efforts of families of cytokines that act as a communication system between cells, especially the subset of chemotactic cytokines known as chemokines. These are approximately 8-10 kd in size and have 4 conserved cysteine residues.

In mammals, the inflammatory response is characterized by the 4 cardinal signs of redness (rubor), heat (calor), swelling (tumor), and pain (dolor). IR, by virtue of its pro-oxidant, free radical action causes tissue damage, and is a pro-inflammatory stimulus (Figure 2). Polymorphonuclear leukocytes, especially neutrophils, are the first population to be mobilized from the bone marrow, where they are stored as mature elements, so their numbers increase rapidly in the circulation even after potentially lethal whole body irradiation (WBI). Within hours, they have transmigrated from blood into tissue lesions. Their exit is followed by bone marrow myelopoiesis that generates more immature polymorphonuclear leukocytes and monocytes. The latter mature into macrophages following their transmigration into lesions; a slower process that takes several days.

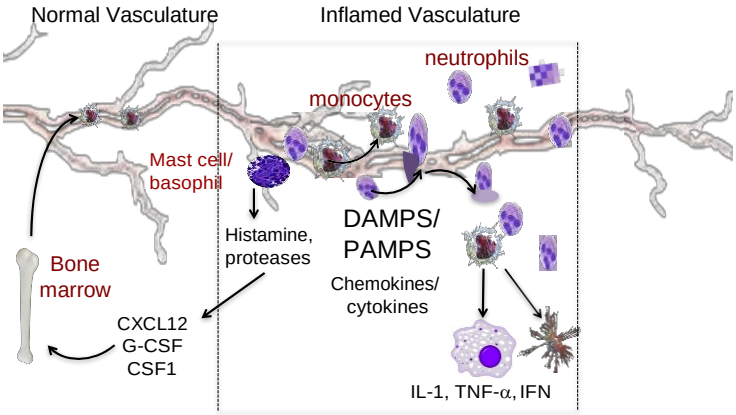

Figure 2: Some of the mechanisms involved in inflammation

The site of transmigration from blood to tissues is marked through the cooperation of endothelial cells, mainly those in microvessels. Initially, the microvasculature contracts through the action of released vasoactive substances like histamine. An interplay is established between locally activated endothelial cells and passing leukocytes that is mediated by pro-inflammatory cytokines, such as TNF-α and IL-1. Under their influence, endothelial cells increase expression of selectins and their integrin ligands, such as ICAM-1 and VCAM-1. Platelets and passing neutrophils are also activated to express integrins that allow them to adhere to endothelium through selectins, causing margination and transmigration of neutrophils between endothelial cells and into the tissue spaces. Further cytokine and enzyme release perpetuates the response.

Endothelial cells separate, blood vessels dilate, blood flow to the site increases, and there is vascular leak with plasma exudation. In extreme circumstances after IR, even red blood cells extravasate. These capillary changes give rise to the redness and swelling of inflammation within a matter of a few hours; a process that is repeated in several waves at various times thereafter. In fact, skin erythema was the first clinical radiation dosimeter The skin unit dose (S.U.D.) or minimum erythematous dose (M.E.D.) was the minimum amount of X-rays to cause reddening 7 to 10 days after exposure. In 1933, Mottram exposed a small portion of the skin of the rat to one M.E.D. and immediately injected a solution of pyrrhol blue into the circulation. He found a blue spot in the radiation site the next day, indicating radiation-induced capillary leak.

Multiple other changes occur in an inflammatory site. Metabolism shifts towards anaerobic glycolysis and the pentose phosphate pathway to provide the energy needed for phagocytosis and detoxification. NADPH is generated and NADPH oxidase, and other free radical generating pathways in mitochondria, cytoplasm, and the plasma membrane, generate reactive oxygen species (ROS) such as superoxide anion, singlet oxygen, hydroxyl ion, and hydrogen peroxide; a response so reminiscent of IR that it forms a second wave of ROS production, the first being over in milliseconds. This “oxidative burst” is particularly strong in neutrophils that also release enzymes, such as lysozyme, proteases; and phospholipases, and cationic peptides, like defensins, to mediate microbial killing. Matrix metalloproteases (MMPs) increase degradation and turnover of the ECM, allowing tissue penetration by myeloid cells. Pro-coagulative and anti-fibrinolytic enzymatic cascades are locally activated to form microthrombi and NETS (neutrophil extracellular traps) made of extracellular DNA bound to a selection of granular and cytoplasmic proteins. These processes serve to further reinforce and expand the pro-inflammatory state of the microenvironment while blood is encouraged to coagulate; a process that has been used in radiation therapy as an emergency method to rapidly stop bleeding in times before the advent of modern coagulative therapies.

These vascular changes have important implications for studies assessing drug or cell delivery and biomarker expression that is often overlooked. Clinically, vascular leak can also have important consequences, especially after potentially lethal doses of WBI or after high regional doses of radiation therapy (RT) where volume effects are marked. Vascular leak is a common indicator of poor prognosis and is often associated with neutrophilia. Experimentally, alterations in plasma volume and selective loss of plasma proteins has major implications for peripheral biomarker studies. Obviously, acute inflammatory events are complex and rapid, proceeding through many series of cascadic reactions. Targeting them specifically can be challenging, but they are an important part of the post-IR scenario and are likely to impact any attempt at radiation mitigation.

PAMPS and DAMPs: Immune recognition of “danger” to the host, and its nature and extent is important for maintenance of homeostasis and tissue function. Molecules from both pathogens and normal tissues are recognized by pathogen- and damage-associated molecular pattern molecules – PAMPS and DAMPs. The best known PAMP is lipopolysaccharide, but many other microbial proteins, lipids, nucleic acids, and polysaccharides act as PAMPS. DAMPS show similar diversity. DAMPS recognized following IR include molecules such as high-mobility group protein1 (HMGB-1), heat shock proteins, ATP, S100a, uric acid, fragments of matrix materials, double-stranded

DNA, RNA, etc, etc. DAMPS may be passively released or actively secreted into extracellular space following damage, or be maintained in an intracellular location, such as the cytoplasm or endosome.

PAMPS and DAMPS essentially utilize similar pattern recognition receptors (PRRs) that may be membrane-associated like the Toll-like receptor (TLR) or C-type lectin receptors, or cytoplasmic, like Nod-like (NLR) and RIG-1-like receptors. TLR form a superfamily with IL-1 receptors, having a Toll-IL-1 receptor (TIR) domain in common. TIR subgroups serve as receptors for interleukins, or PAMPS, or adaptor signaling molecules. A subset of NLRs with other molecules form multi-molecular structures called inflammasomes that can activate caspase 1 and convert pro-IL-1 and IL-18 into mature cytokines, in so doing playing a major role in inflammation. Different PRRs are activated by different stimuli, are expressed differentially by different cell types, and activate different signaling pathways. So, in the irradiated gut, both microbial and damage-related products will stimulate both epithelial and gut-associated lymphoid tissue to respond through essentially similar pathways, depending on receptor expression. The overall result is further production of cytokines, such as type I interferons (IFN), TNF-α, IL-1, IL-6, and IL-18 that in general tend to be pro-inflammatory, although anti-inflammatory molecules such as IL-10 may also be released.

IR Effects on Innate Immune Systems: There is increasing evidence in the literature that IR suborns TLR signaling pathways, in some cases dovetailing with a classic DNA damage response to increase TLR expression on the membrane, in others signaling through cytoplasmic pathways. Perhaps the most significant danger signal after IR is dsDNA. The AIM2 inflammasome recognizes dsDNA, as does the cGas-STING pathway. Cytosolic dsDNA can be formed after IR exposure, most often in the form of micronuclei, or after some other breach in the integrity of the nuclear membrane. In fact, there are many possible sources of cytoplasmic dsDNA, especially if DNA repair is defective, or senescence a likely radiation consequence, and different pathways may be utilized. It is thought that these DNA recognition mechanisms evolved primarily to recognize viral nucleic acids, since the predominant output is type 1 IFN. After IR, micronuclei only form only after mitotic progression so they may be most important in proliferative tissues, but they can persist for several cell cycles so they may play a role in chronic inflammation. As late responding tissues are forced into proliferation late, the appearance of micronuclei may be delayed. As a result, dose will be important in terms of what pathway is utilized. As Puck and Markus classically demonstrated, cells that undergo mitotic death can replicate for 1-3 generations after exposure to doses up to about 8 Gy before dying. After doses higher than about 8 Gy many cells will not progress through mitosis and PRR pathway utilization may change. For example, IR induces TNF-α rapidly after exposure in non-mitotic and mitotic cells but only after relatively higher radiation doses.

Overall, the fact that IR is a pro-inflammatory signal means that it generates sterile inflammatory lesions that resemble those stimulated in response to pathogenic invasion. Tissue damage can be a direct result of radiation dsDNA damage or be caused indirectly through innate immune cell activation and infiltration into irradiated tissues, which can cause secondary ROS, DNA damage, and cell death. In these ways, the sphere of influence of IR exposures can extend beyond direct target cell killing into local, regional, and systemic effects, including what has become known as bystander effects.

Importantly, “danger” signaling pathways link innate to adaptive immunity. They do this by causing maturation of subsets of innate immune myeloid cells that have phagocytosed antigens into dendritic cells (DC) that can process antigenic peptides for presentation to lymphocytes so as to generate adaptive immunity. Through generating DAMPS and causing inflammation, IR can mature DCs and affect pathways of antigen processing. Thus although IR is generally thought of as being immunosuppressive, it is better considered as an immune modulator. These aspects of the adaptive immune response are modulated by IR.

C. Adaptive Immunity:

The major difference between innate and adaptive immunity is that the latter is induced by Ag and mediated by classic T and B lymphocytes that respond specifically to antigenic determinants and develop immunological memory. It is true that evidence has recently emerged showing that innate immune cells can retain some memory of exposure, but this is more adaptation than true immunological memory. It is also true that lymphocytes can display innate immunity, as is the case for NK or NKT cells, but these are not antigenic peptide-specific.

A prerequisite for antigen-specific lymphocyte responses to be generated is presentation of Ag, which is a property expressed by several cell types, but the most professional are dendritic cells (DC), which themselves can be of several types, including Langheran’s cells in the skin. DCs present peptide Ag in the context of MHC molecules; class I for CD8+ cytotoxic T cells and class II for CD4+ T cells. CD4+ T cells cooperate with other immune populations (T, B, or myeloid) to control their responses, but can be considered as being either helpers or regulators. They come as various subsets that mediate distinct functional aspects of adaptive immunity (Figure 3).

The major conventional DC type is of the myeloid series. DCs take up and degrade native proteins into peptides for loading onto MHC molecules; the complex being recognized by specific T cells via antigen-specific *αβ* T-cell receptors. If Ags are taken up exogenously and presented on MHC class I molecules, it is known as cross-presentation, which can be by 'cytosolic' or 'vacuolar' pathways. In the former, more prominent pathway, degradation is by the proteasome with peptides transported to MHC molecules by transporter associated with antigen processing 1 (TAP1) and TAP2 molecules. Peptide loading occurs predominantly in the endoplasmic reticulum for class I molecules, with peptides of around 9 amino acids, and in endosomes for class II molecules with peptides of a slightly larger size. By contrast, the vacuolar pathway does not involve the proteasome or TAP, but is sensitive to inhibitors of lysosomal proteolysis, which is a

limited process in DCs. Activated CD8+ cytotoxic T cells (CTL) recognize class I–peptide complexes on non-immune cells to effect lysis.

Presentation of antigen for the generation of immunity requires maturation of DCs; a process that “licenses” DCs to stimulate T cells. This interaction takes place within what is called an immunological “synapse (Figure 4). Without this maturation process DCs

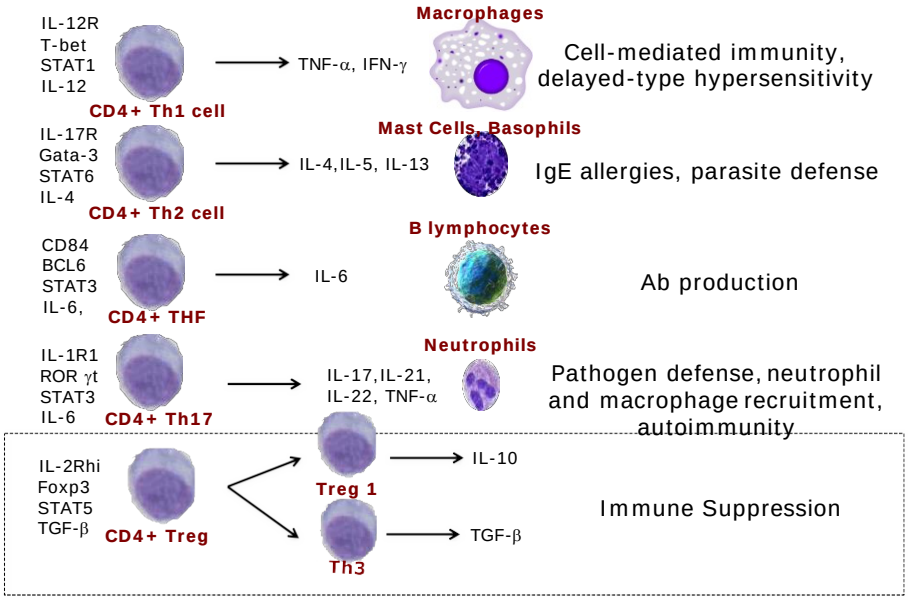

Figure 3: CD4+ T helper and regulatory cells, showing their expression of cytokine receptors, transcription factor and signaling pathway utilization, and cytokine production (left), interacting cells, and functions (right).

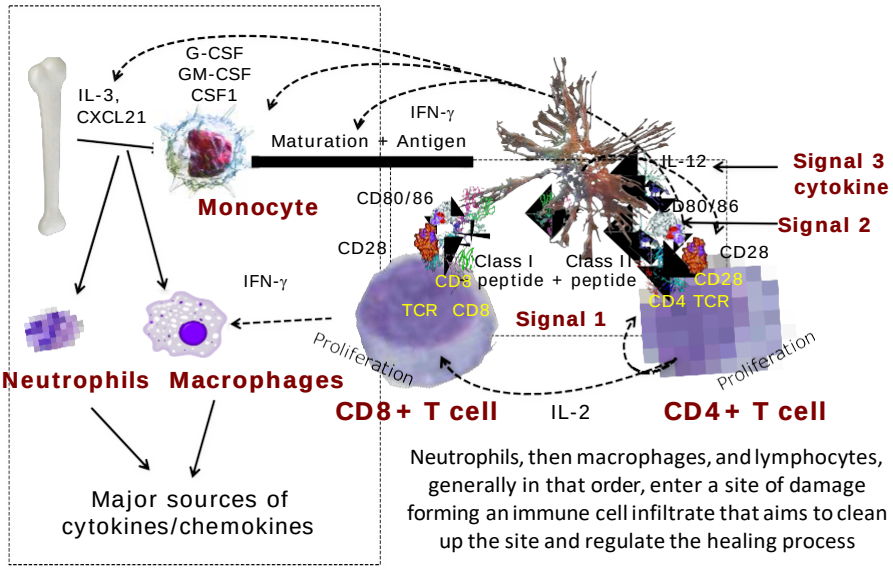

Figure 4: The immunological synapse, showing DC interacting with CD8+ (left) or CD4+ T (right) cells, the interaction between the TCR and MHC+Ag (Signal 1) Cd80/86 and CD28 (Signal 2) and cytokines released (signal 3) with feedback to bone marrow.

induce peripheral tolerance rather than immunity. The first step is binding of the T cell receptor (TCR) to the Ag-MHC complex, which is known as signal 1. However, with maturation, DCs gain CD80/86 (a.k.a. B7) co-stimulatory or co-inhibitory molecules that also bind a CD28 or CD152 (CTLA4) on T cells, which acts as a second signal for T cell activation. Stimulatory cytokines, such as IL-12, act as signal 3 to more optimally activate T cells. Antigen-specific proliferation with IL-2 expands the responding cells, so a single naive CD8 T cell can end up producing as many as 10,000 daughters.

Figure 3 serves to illuminate some of the diversity of immune responses, as reflected by CD4+ T cell subpopulations, which is much greater than within CD8+ cells. Some of these responses result in tissue damage, for example through the action of cytotoxic CD8+ cells or M1 macrophages, which are activated and programmed under the influence of cytokines released by activated Th1 or Th17 cells. In general, it is the cell types that mediate various forms of cell-mediated immunity that cause tissue damage. However, it can also be caused by any of the 3 types of antibody-based allergic reactions (type I to III hypersensitivity), and by activated innate immune myeloid and lymphoid effector cells. Clearly, a delicate balance must be established that is sufficient to effectively carry out effector function without excessive damage. One mechanism that limits damage is seen in chronic inflammatory conditions caused by viruses, bacteria, and tumors - it is functional T cell exhaustion. Exhausted CD8 T cells exhibit a genetic signature that is unique compared with either effector or memory CD8 T cells. The do not express CD62L or CCR7, or CXCR3, and are therefore impaired in lymph node trafficking and localization. They can however be reactivated by short term culture in IL-2 to display highly effective memory and effector cell function. Various other controls are in place to limit immune damage, including T regulatory cells, negative immune checkpoints (figure 4), immunosuppressive myeloid cells and cytokines. A major issue in RT is to what extent these checks and balances are disturbed by IR.

IR Effects on Adaptive Immunity: The high radiosensitivity of lymphocytes was one of the first radiobiological observations, and reported by Heineke in 1903. This radiosensitivity refers primarily to resting lymphocytes that die rapidly by an apoptotic process. In fact, on activation, and with gain in effector function and loss of proliferative ability, lymphocytes gain radioresistancy, so not all lymphocytes are equally radiosensitive. These differences are important as they are reflected in the relative impact of IR on different aspects of immunity. In general, pre-existing immune function is little affected by IR – it is the precursor cells that are sensitive. So, NK cell function is radioresistant but the generation of NK cells is radiosensitive. Antibody production by plasma cells is radioresistant, but their progenitor B cells are radiosensitive. Cytotoxic and helper T cell functions are radioresistant but their generation is radiosensitive. An exception may be T regulatory cells that are relatively radioresistant, although North showed many years ago in a tumor model that 5 Gy whole body irradiation within a narrow time window after tumor transplantation could eliminate this population causing tumor regression. However, the existence of “natural” and “induced” T regulatory populations blurs the issue, and North was clearly dealing with the induced population. Different lymphocyte lineages also appear to vary in radiosensitivity with B cells and CD8+ cells being generally more radiosensitive than CD4+ cells. Finally, antigenic stimulation affects radiosensitivity. In general, while antibody production and immunological memory are relative radioresistant,

the timing of antigen exposure relative to radiation exposure is critical. At least for antibody production, if antigen is given after WBI, responses are suppressed, but if given even a short time before they are resistant and may be even enhanced. Such increases may be due to compensatory hyperplasia following radiation-induced lymphocyte depletion, which has been exploited in conditioning regimens to enhance proliferation of adoptively transferred T cells for cancer immunotherapy.

The rapidity and extent of death of resting lymphocytes remains the simplest biodosimeter of WBI exposure. It is also seen after local RT, as blood passes through the radiation field. As Shohan noted in 1916 “Every time we roentgenize any given area, we thereby also radiate the entire volume of blood.”

D. Organization of the Immune System

“Primary” lymphoid organs are the sites of lymphocyte development. For B lymphocytes, this is the bone marrow and, for T cells, the thymus. Adult thymectomy followed by WBI and bone marrow transplantation results in an almost complete lack of T cells, which is also the case in nude (nu/nu) mice that are born with no thymus. In these developmental sites each lymphocyte comes to express receptors with specificity for only one antigenic determinant, i.e. they become clonal. Those with specificity for self and those unable to recognize self MHC are eliminated during development, resulting in a state of “central tolerance”. Lymphocytes are exported from these sites “secondary” lymphoid organs that include the spleen and lymph nodes where most responses are generated.

The immune system can simplistically be considered as being composed of mobile cells and tissue residents, although there is considerable plasticity in phenotype and function, especially among myeloid cells. For example, inflammatory myeloid-derived monocytes can differentiate into conventional DCs, effector inflammatory macrophages (M1), or alternatively activated, anti-inflammatory M2 macrophages. In addition to these bone marrow-derived myeloid populations, tissues contain resident macrophages that are self-renewing and embryo-derived rather than coming from hematopoietic tissues. Resident macrophages primarily fulfill tissue-specific and niche-specific functions, for example clearance of cellular debris, response to infection, and resolution of inflammation. Mobile and resident myeloid subsets express different endocytic and pattern recognition receptors and secrete different cytokines and chemokines following activation. In a site of inflammation, all subpopulations may co-exist. Tissue resident lymphocyte populations also seem to exist, although their relevance is frequently obscure. The general conclusion that different tissues contain resident immune populations that are characteristic for that particular tissue is however inescapable, and is most obviously true for mucosal associated lymphoid tissue (MALT) and the skin immune system.

The development of effective and regulated immunity relies upon co-ordinated migration of different cellular components. This occurs under normal steady-state conditions, but is heavily influenced by diverse signals that come from a disturbed tissue microenvironment. One normal patrol pathway for naïve T cells is to exit the blood via high endothelial capillary cells in the lymph nodes, flowing through lymph to the thoracic duct and

recirculating back into blood. This transit through secondary lymphoid organs establishes T cell dependent areas, such as the periarteriolar regions in the spleen and the cortex of the lymph nodes, that are noticeable by their lack of cellularity in nude mice. In fact, perhaps 90% of resting lymphocytes are in transit at any one time, and only 10% of these are in the blood. This recirculation pathway enables naïve T cells to contact antigen brought to the peripheral lymphoid structures by DCs, normally as a result of their migration from inflamed sites. Responses are generated in these peripheral organs over a period of at least several days, with resultant swelling and trapping of recirculating cells. Once generated T cells are exported and return from the blood to penetrate the site of inflammation where they develop into effector cells. Antigen-specific T cells are selectively retained in the site, resulting in their proportional enrichment. This is easily seen by the higher percent of tumor antigen-specific T cells in tumor infiltrating lymphocyte populations. The fact that RT and other therapies often deplete peripheral lymph nodes in the field and affect lymphocyte recirculation to the tumor suggests a negative influence on the development of local immunity, although the importance of this for the therapeutic outcome has yet to be fully established.

To generate a state of systemic immunity that can mount fast and effective re-challenge responses in any affected tissues, lymphocytes must generate “memory”. This involves not only clonal expansion after contact with antigen but the development of homing phenotypes that can withstand the rigors of trafficking throughout the body. Phenotypically, antigen-experienced T cells can be subdivided into central memory (T_{CM}), effector memory (T_{EM}) and effector (T_{EFF}) cell subsets, although these distinctions are not sharp and plasticity commonly occurs, especially in non-steady state conditions. Effector T cells generally are poor at homing, and are short lived, with 90–95% of the responding T cell population undergoing mass apoptosis following the peak expansion phase. However, an estimated 5–10% survive this contraction phase and give rise to long-lived memory cells populations that express CCR7 that allows them to home to secondary lymphoid tissues. In other words, as T cells transition from naive to effector to memory cells, the overall gene expression profiles change to endow each population with unique characteristics and/or functions, including homing and trafficking. In this way, memory T cells freely circulate between the blood, spleen, lymph nodes and populate many peripheral tissues (but not all - such as the skin and gut); they can be rapidly recruited to localized areas of inflammation without antigenic restimulation. The memory:effector cell ratios can be modulated, for example by the immunosuppressive cytokine TGF-β, and modulating this ratio could have great relevance to cancer immunotherapy. The involvement of T regulatory cells that are critical for maintenance of peripheral tolerance and controlling the expression of immunity also affect these ratios. In the absence of T regulatory cells, autoimmunity is more prevalent and potentially lethal cytokine storms become a distinct possibility.

IR Effects on Organization of the Immune System: There is little doubt that IR disturbs the organization of the immune system, but the implications and consequences are often hotly debated. IR rapidly decreases the sizes of the thymus, spleen and lymph nodes by killing lymphocytes. Extracorporeal irradiation, for example of the spleen, decreases the recirculating T cell pool over time. The stroma in immune organs are however relatively radioresistant, for example, the thymic epithelium that is critical for T cell development

and recovery. After modest radiation doses, the thymus becomes repopulated and the T cell pool regenerates over time. However, the time between irradiation and commencement of sustained regeneration is dose-, tissue-, and species-dependent, with humans tending to show prolonged depletion even after local RT. The same is true for the bone marrow, which is the stem cell source of all lymphocytes in the adult. In rodents, bone marrow lymphocytes diminish within 4-5 days after doses of the order of 300 cGy WBI; recovering after a few weeks. The thymus may recover slightly earlier. The recirculating T cell pool is depleted in two phases, with first rapid disappearance of small lymphocytes followed by slower loss of other cells. Regeneration may not be complete even after 3 months. In general, the degree of lymphoablation needed for graft acceptance or functional suppression of cell mediated immunity is higher than one would expect based purely on depletion of cell numbers.

E. Acute and Chronic Immune Responses after IR

The initial biological responses to the physico-chemical actions of IR are very rapid. In addition to the classic DNA damage response, levels of extracellular ATP, ADP, GSH, glutamate, NADH, and other small cytoplasmic metabolites increase in the external milieu. Many of these can be considered as DAMPs. P2X (ligand-gated ion channels) and P2Y receptors (G-protein–coupled receptors) are intimately involved in the export processes and subsequent fluxes, as are other receptors and enzymes, such as those related to production of adenosine, which is a highly immunosuppressive rapid response element. The internal changes in redox following IR can have direct effects on molecules and structures controlling important signal transduction pathways. This includes pathways under the control of transcription factors such as Nrf2, NF-κB, Ref-1, TXN, AP-1, and structures such as the 26S proteasome, which is a redox-sensitive target that disassembles and reassembles in response to oxidative stress to effect multiple changes, including changes in metabolism.

Together, by a host of what appear mysteriously co-ordinated processes, transcriptional and post-transcriptional/post-translational responses transition into an archetypical early gene response. Here, we are talking about the first few hours after exposure, prior to outcomes such as apoptosis, mitotic cell death, autophagy, senescence, etc.. This initial phase is primarily a “danger” response that has acute inflammation and the innate immune system as central effector elements, but extends to influence DNA repair, cell cycle arrest, and other responses familiar to radiobiologists. Since this is a co-ordinated response involving many diverse elements, the dose response for any individual component is often non-linear, varying with time, dose rate, radiation quality, cell type, tissue, and species. The ultimate primordial purpose of this danger response is to alter the tissue environment so as to support an influx of immune cells and the elimination of pathogens. This inevitably involves considerable collateral damage, even though the site is sterile, as after irradiation. This dualism – the need to cause damage to deal with “danger” while at the same time working to restore homeostasis – is an inherent feature of the immune system that involves the co-existence of both pro- and anti-inflammatory forces under redox control.

The balance of opposing forces allows inflammation to be localized, minimizing systemic toxicity, which can be life threatening and a concentration gradient to be established for chemotaxis of responding immune cells. Acute phase proteins, which are largely serum biomarkers of inflammation, are made largely in the liver. DAMPS also are released and can work as biomarkers of exposure. Some DAMPS, such as HMGB1 are pro-inflammatory, but anti-inflammatory feedback control mechanisms kick in with time to dampen the damage caused and, in time, to direct immune responses towards healing.

For pathogens, failure to eliminate them as a source of stimulation results in a chronic inflammatory state with continuous immune cell infiltration. The distinction between acute and chronic inflammation is often considered to be the presence of lymphocytes in the latter, but this underestimates the complexity of these conditions, which vary enormously. As can be seen from figure 5, brochoalveolar lavages of fibrosis-sensitive C57Bl/6 mice and pneumonitis-sensitive C3H/HeN show repeated waves of immune infiltration after lung irradiation, with dramatic early and late changes in lymphocytes. Similar patterns are seen after lethal and non-lethal radiation doses (bottom left). While bronchoalveolar lavage does not necessarily reflect events within the lung tissue itself, cytokine mRNA assays for the whole lung (Figure 6) clearly indicate similar waves of cytokine expression that implicate myeloid cells and TNF-α in pneumonitis, which seems controlled in the C57Bl/6 strain that develops fibrosis.

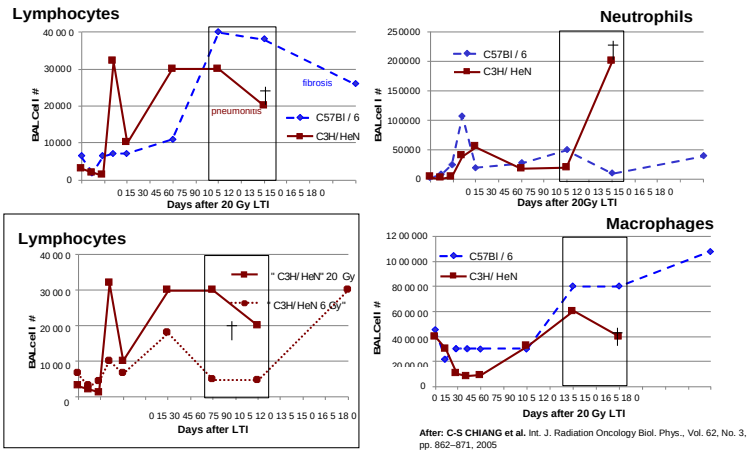

Figure 5: Immune infiltrates in bronchoalveolar lavage of mouse at various times after thoracic irradiation of fibrosis resistant C57Bl/6 and pneumonitis sensitive C3H/HeN mice.

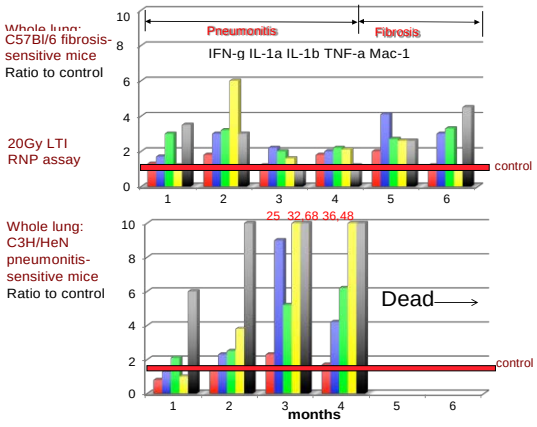

Figure 6: Cytokine mRNA levels in whole lung derived from the same study as figure 5.

The waves of inflammatory cells and cytokines can translate into clinical effects. This was recognized by radiobiologists even prior to 1920. They noted that irradiation induced waves of skin inflammation in humans and rodents that could be related to dilatation of microcapillaries. Waves of proinflammatory cytokines similar to those in figure 6 occur in brain and probably all tissues after RT, and their timing can be related to clinical changes. Mortality waves are seen in murine survivors of WBI, and this seems unaffected by bone marrow transplantation. The sources that contribute to these waves may be multiple, but the fact that periodicity increases with time suggests that at least some are related to cell turnover within the tissue. The relationship between tissue turnover and the expression of radiation damage was another early radiobiological observation, with more rapidly proliferating tissues being classified as “early responders”. This does not rule out a role for radiation-induced cytoplasmic DNA as a driving force. Indeed, if anything it supports the view that this DNA is generated by cell proliferation and may be associated with autophagy and senescence.

ARS mortality is a major focus of mitigator research, especially those syndromes classically ascribed to failure of hematopoietic or intestinal systems. The classic in situ clonogenic survival assays that were developed to assess damage and recovery in these tissues have formed a backbone for radiobiological research for the last 50 years. Functional failure correlates well with tissue turnover times. On the other hand, the finding that mitigators are effective when given 24 hours or more after IR exposure, indicates that more than the initial IR-induced response may modify the rate of stem/progenitor cell regeneration in these tissues. The immune system is an obvious target in this respect and classes of mitigators exist that appear to act primarily through anti-inflammatory pathways, although other pathways, such as those that stimulate proliferation to accelerate regeneration, are also effective.

The role of stem/progenitor cells viz-a-viz inflammation is less clear in late than acute responding tissues. The former proliferate slowly and express damage late. Clonogenic assays have been correlated with tissue failure for kidney and spinal cord, but many late responses are better considered under the general rubric of life shortening, which is a well-known consequence of low dose WBI exposure. For example, A-bomb survivors have significantly increased, dose-related incidences of late non-cancerous conditions including thyroid and liver disease, cataracts, glaucoma, hypertension, and myocardial infarction, and disturbed immune homeostasis. Similarly, survivors of the Tokai-mura criticality accident succumbed to a mixture of chronic hematological and late-onset non-hematological complications, and individuals exposed to waterborne uranium fission products from the Mayak facility developed an increased incidence of various chronic diseases. A clinical parallel is in childhood cancer survivors treated with WBI plus bone marrow transplantation who have a higher, but variable, incidence of radiation pneumonitis, veno-occlusive disease, renal hypertension, cardiac damage, neurocognitive abnormalities, cataracts, endocrine dysfunction, and hormonal deficits. Multiple organs are involved. In mice, morbidities and mortalities seem similar after WBI with or without bone marrow rescue and increases in type 2 diabetes and cardiac disease have been reported in non-human primates.

Data on late effects of IR exposure suggest that much late morbidity, and even mortality, can be ascribed to chronic inflammatory reactions, expressed as diverse symptoms in

one or multiple organs. The context suggests that late symptoms of WBI fall under the general rubric of multi-organ disease syndrome (MODS) and overlap with those associated with premature aging or increased frailty exemplified by chronic inflammation, cardiovascular disease, and metabolic syndrome. These immune imbalances are particularly associated with a shift towards activation of cells of the myeloid system. This lymphocyte to myeloid shift has important implications. The immune system is frequently found to be dysfunctional late after radiation exposures. A major question that remains unanswered is whether this imbalance is due to lymphocyte cell loss or to myeloid cell suppressive influences.

The emerging concept is that the immune system is reactivated at various times following IR exposure, as seen by the wave-like patterns of cytokine production, and that this is related to tissue turnover and most likely cytoplasmic DNA levels. One consequence of these waves is a shift towards chronic activation of the myeloid system, and a long-term disturbance in immune homeostasis that may express itself in different ways. For example, after LTI, C57Bl/6 mice appear able to control the inflammatory circuits, primarily in myeloid cells, that generate pneumonitis; but they may succumb to pulmonary fibrosis (Figure 6). In addition, different organs may be involved in different animals. The reasons for this are obscure, but a likely explanation is that vascular damage leads to an inflammatory nidus and that this is essentially a systemic event occurring more or less at random in different tissues; which brings us back to the beginning of this article.