Published in final edited form as:

Cancer Lett. 2015 November 28; 368(2): 238–245. doi:10.1016/j.canlet.2015.03.031.

Radiation takes its Toll

Josephine A. Ratikan, Ewa D. Micewicz, Michael W. Xie, and Dörthe Schaeue*

Department of Radiation Oncology, David Geffen School of Medicine, University of California at Los Angeles, CA, USA

Abstract

The ability to recognize and respond to universal molecular patterns on invading microorganisms allows our immune system to stay on high alert, sensing danger to our self-integrity. Our own damaged cells and tissues in pathological situations activate similar warning systems as microbes. In this way, the body is able to mount a response that is appropriate to the danger. Toll-like receptors are at the heart of this pattern recognition system that initiates innate pro-oxidant, pro-inflammatory signaling cascades and ultimately bridges recognition of danger to adaptive immunity. The acute inflammatory lesions that are formed segue into resolution of inflammation, repair and healing or, more dysfunctionally, into chronic inflammation, autoimmunity, excessive tissue damage and carcinogenesis. Redox is at the nexus of this decision making process and is the point at which ionizing radiation initially intercepts to trigger similar responses to self-damage. In this review we discuss our current understanding of how radiation-damaged cells interact with Toll-like receptors and how the immune systems interprets these radiation-induced danger signals in the context of whole-body exposures and during local tumor irradiation.

Keywords

Toll-like receptors; Radiation; Reactive Oxygen Species; Redox; Inflammation

1. Pattern recognition and immunity

For a long time, innate immunity was seen as the primitive and separate entity to the much more sophisticated and powerful adaptive arm of the immune system. This view changed drastically with the discovery of the mammalian homolog to the cytosolic domain of *Drosophila melanogaster*'s Toll protein [1]. Now we know that the Toll/IL-1R/resistance protein (TIR) domain is an intricate part of every member of the Toll-like receptor (TLR) family on innate immune and other cells and crucially links the two arms of immunity, as originally suggested by Charles Janeway Jr.[2]. Germline encoded TLRs and other pattern

*Corresponding Author: Dr Dörthe Schaeue, Department of Radiation Oncology, University of California at Los Angeles, 10833 Le Conte Ave, Los Angeles, CA 90095-1714, USA, Phone: 001-310-825-5069, Fax: 001-310-206-1260, dschaeue@mednet.ucla.edu.

Conflicts of interest

none

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

recognition receptors (PRRs) have evolved to recognize conserved pathogen-associated molecular patterns (PAMPs) encountered during an infection (or PAMPs have evolved to fit PRRs, in Matzinger's hypothesis [3]). The basic assumption is that without the TLR recognition system acquired immunity would not have evolved [4], and the importance of this discovery was recognized by the Nobel Prize committee in 2011 by awarding Jules A. Hoffmann, Bruce A. Beutler and Ralph M. Steinman.

Perhaps one of the most surprising features of TLRs – especially of TLR2 and TLR4 - is, that these receptors also recognize endogenous danger molecules that appear following tissue damage, so-called damage-associated molecular patterns (DAMPs) [3, 5–9]. The diversity of PAMPs and DAMPs that are recognized by TLRs is considerable (Table 1) [10–13] but what unifies them is their ability to signal 'Danger' to the immune system and they are now central to the danger theory [3]. Antigen presenting cells, dendritic cells in particular, integrate these danger signals and undergo an important maturation process where they acquire the ability to present T cells with co-stimulatory elements (signal 2) alongside cross-presenting the antigen (signal 1) and a 3rd signal (cytokine signal) that together initiate adaptive immunity [14–16]. Conversely, in the absence of TLR signaling, DCs remain immature and will not relay this crucial signal 2, but rather make T cells anergic or maintain a tolerant state [17]. TLR signaling generally also aids macrophage polarization towards the pro-inflammatory M1 phenotype that feed forward towards immune activation [18]. PRRs other than TLRs that tend to deal with more restricted ligands have also evolved. These include C-type lectin family receptors (Dectin-1), cytosolic retinoic acid-inducible gene-I-like helicases (RIGs such as MDA-5) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) i.e. inflammasomes. These will not be discussed here in any detail, but recognition of nucleic acids is likely to make critical responses following radiation damage as well as in virus recognition.

2. Toll like receptor signaling

Today there are 10 human TLRs that we know of, and 2 more in mice [12]. They are predominantly found on innate immune cells (polymorphs, macrophages and DCs) but B cells and some T cells have them too, as do many non-immune cells, e.g. endothelial and epithelial cells [19, 20]. Ligand binding to leucine rich repeats triggers receptor dimerization and initiates signaling pathways via the TIR domain. Common adaptor proteins for TIR are myeloid differentiation primary-response protein 88 (MyD88), MyD88-adaptor-like protein (MAL), TIR domain-containing adaptor-inducing interferon- β (TRIF) and TRIF-related adaptor molecule (TRAM). Signaling downstream of these adaptor molecules drives IL-1R-associated kinases (IRAKs) and TNF receptor-associated factor (TRAFs) that tend to feed into two distinct avenues: the activation of 1) pivotal inflammatory transcription factors nuclear factor kappa B (NF κ B), cAMP response element-binding protein (CREB) and activator protein 1 (AP-1) or 2) interferon—regulatory factors (IRFs). As a result, pro-inflammatory cytokines and/or Type I interferons are induced. Which of these two avenues is pursued seems to depend partly on where the TLR ligand was detected in the first place: extracellular ligands binding to TLR2/TLR1, TLR2/TLR6, TLR4, TLR5 or TLR11 tend to push primarily a NF κ B pro-inflammatory cytokine pathway while intracellular, endosomal TLR engagements (TLR3, TLR4, TLR7-8, TLR9, TLR13) open the type-I IFN route with

some potential crossover between these paths. This is perhaps best exemplified by the complex, localization-dependent switch in signaling of TLR4 – the last puzzle in the ever-elusive LPS receptor. Extracellular LTR4 engagement drives NF κ B but endosomal TLR4 activation adds type-I IFN to the equation. Clearly, the potency and the diversity of the inflammatory and immune activating cascades downstream of TLRs is enormous [21, 22], illustrated by the fact that deficiencies in TLR signaling can lead to serious, even life-threatening diseases [4].

The infiltration of immune cells that TLR signaling generates, initially innate players such as polymorphs then monocytes, possibly followed by lymphocytes, drives a pro-inflammatory, pro-oxidant environment – i.e. a bona fide inflammatory response and eventually turns it into one that will allow resolution of inflammation, tissue repair, regeneration and healing [21, 22] and/or in the case of lymphocyte involvement, immune reactivity. It is in its nature for an inflammatory response to cause additional tissue damage - at least initially- through excessive Reactive Oxygen or Nitrogen Species (ROS/RNS) production, proteolytic enzyme activity and self-perpetuating cytokine loops [22–24]. This is the point of contact with radiation that generates ROS/RNS, a response that is perpetuated by pro-inflammatory cytokines further driving ROS/RNS production including superoxide anion, hydroxyl radicals, hydrogen peroxide, nitric oxide, peroxinitrite and others [25, 26]. Redox-sensitive signaling pathways are activated, that may perpetuate tissue damage and even cause cell death or the reverse, if the response switches to be anti-oxidative [22, 27]. This will depend upon the extent of the feedback. For example, TLR signals can lead to NADPH oxidase activation and ROS production [28] but ROS in turn can prime for further TLR activation in a self-perpetuating loop that can be broken by antioxidant *N*-acetylcysteine (NAC) [29]. Ultimately, the danger signals need to be removed so that homeostasis can be restored.

3. Radiation damage, DAMPs and TLR activation

The concept that radiation signals danger to the immune system through TLRs and other PRR is attractive [30] and has received much attention recently both clinically and preclinically [5, 31–34]. The assumption is that radiation-damaged cells release DAMPs that bind to TLRs (Table 1) and hence activate canonical inflammatory pathways and/or initiate immunity. The chromatin-binding nuclear protein high-mobility group box 1 (HMGB1), a highly-conserved non-histone DNA-binding protein that fits onto TLR2 and TLR4 and subsequently drives NF κ B and ROS production, is one such example [5, 35, 36], as are small and large RNAs activating TLR3 [37]. Other DAMPs released from irradiated cells and tissues include DNA, ATP and UTP, chromatin, histones, mitochondrial DNA, heat shock proteins, calreticulin and other cell components [32, 38–43]. Mitochondrial DNA is known to activate TLR9, as does chromatin after forming complexes with IgG [44, 45], but not all radiation-induced DAMPs signal through TLRs. They can use alternative PPRs instead.

The time course and magnitude of DAMP release may vary for each signal, but they are likely to cooperate and amplify the overall warning sign to the body. For instance, TLR-NF κ B-driven synthesis of IL-1 β can prime a TLR-independent sensing of ATP and/or DNA

that licenses the inflammasome to yield yet more IL-1 β through posttranslational processing [46, 47]. The role of inflammasomes in radiation-induced responses is only beginning to be appreciated [48] but we have certainly known for some time that irradiated tissues often show a very strong IL-1 β signal [49, 50]. ATP release and its binding to purinergic P2 receptors tends to be a rapid process [51, 52], while mitochondrial DNA and peptides and dead cell components are likely to appear later on and signal more intensely, even causing a systemic response syndrome if the tissue damage is severe [53]. Once outside the cell, these molecules are faced with a much more oxidizing environment and therefore redox-specific moieties make especially potent DAMPs [54]. Surprisingly, HMGB1 can act as a DAMP only under reduced conditions, whereas it is being immunologically ignored when oxidized for instance during some forms of apoptosis [55]. As difficult as it is to quantify, a crucial determinant in tissue damage responses and in directing the immunogenicity of cell death is redox [56].

All in all, concrete experimental evidence for TLR activation following radiation-driven DAMP release remains relatively limited at this point. Of note, DNA damage and ATM activation can drive ligands for another innate receptor, namely the NKG2D receptor that is particularly relevant for NK cells activity [57] and it is easy to extrapolate this and envision a similar scenario for a radiation-driven TLR activation. Indirect evidence in support of this comes from the observation that even immature DCs can induce anti-tumor immunity when injected into an irradiated tumors indicating that the irradiated microenvironment must have provided sufficient danger i.e. maturation signals [58].

What's more, the TLR gene family may be integrated in cellular radiation responses in alternative ways to the one mentioned above. Certainly, the work by Menendez and colleagues is groundbreaking as it suggests that TLR gene expression in human lymphocytes is part of the radiation DNA damage response and is largely p53-dependent [59], perhaps through a p53 consensus site in the TLR promoter [60]. Of note, murine TLR genes lack the necessary p53 response element and do not show obvious TLR gene induction under similar conditions. The fact that 18 different individuals didn't necessarily exhibit identical TLR response profiles -qualitatively nor quantitatively- points to the unique and personalized nature of the human immune system that we are familiar with. More importantly and not surprisingly, a measurable downstream consequence of this DNA-damage driven TLR gene induction is the engagement of the cytokine network, again variable in nature and magnitude for each individual. Ultimately, this will shape one's innate immune response with potentially far-reaching consequences if we consider the intricate link to adaptive immunity. Such local innate immune signals that are downstream of DNA damage may also relate to the phenomenon of adaptive responses to (same or different) systemic stressors by enhancing protein turnover [61], and point at evolutionary systems in multicellular organisms geared towards survival in unfavorable environments [62]. What this also tells us is that radiation responses in vivo are likely to engage the immune system in more than one way and that we are only beginning to appreciate this complex interplay.

4. First come, first served

How multiple TLR signals convey different messages seems to depend in part on the order they are received. We know this from our own experience when combining radiation with the TLR2/4 agonist LPS, where radiation is able to prime cells for increased LPS-induced cytokine release when given before LPS, but less so when given afterwards [63]. This is certainly plausible considering a radiation-induced TLR gene induction would, for a start, increase a cell's readiness to bind LPS. Indeed, this is also what Menendez et al. [59] observed, at least when using the p53 inducer, nutlin, to drive TLRs prior to exposing cells to a TLR2 agonist. Taking this further, one may view the immune system and TLRs in particular as sensors and amplifiers of radiation damage. We strongly believe that cells have an internal oxidant/anti-oxidant rheostat, that, if tilted towards excessive ROS upon -let's say- a radiation insult, then our anti-oxidant defense under the control of Kelch-like ECH-associated protein 1 (Keap1) / NF-E2-related factor 2/ (Nrf-2) and Heat shock factor (HSF-1) / Heat shock proteins (HSPs) kick in to correct that by driving an anti-oxidant/anti-inflammatory response (Figure 1)[64]. However, if the initial radiation dose is small, the ROS/DAMP levels may not rise above a certain threshold, and fail to activate the compensatory anti-oxidant response [34, 35, 65]. However additional PAMPs, e.g. LPS, under such circumstances may push the ROS levels high enough for the damage to be seen because ultimately PAMPs and DAMPs roll on the same internal rheostat.

Where, within the dose range, this threshold falls is not entirely clear and will likely depend on the organ/tissue studied and the experimental system used. According to Menendez [59], 4Gy seems sufficient to do so, which is in line with our experience on Nrf2 activation at similar dose levels but even then cells are very efficient at mopping up any excessive ROS, at least initially [64]. Similarly, downstream inflammatory networks are not usually engaged in vivo unless doses of 7Gy or higher are given [66] which is reminiscent of the fact that low radiation doses of about 1.5–3Gy given in 0.3–0.5Gy fractions can be surprisingly anti-inflammatory in an already-inflamed environment [67]. Of note, TLR activation by radiation doses of as low as 0.05Gy can be detected in vitro [35] but whether they are relevant in vivo, for instance during CT scans at such dose levels, needs to be evaluated. Certainly, one could argue that this may become pertinent when additional, amplifying TLR signals are at play, let's say from the gut microbiome (see below). Further, if and when such a radiation-driven TLR signal maybe saturated is unclear, but giving doses in excess of about 10–20Gy are unlikely to further enhance this signal [33], especially in vivo where immune-regulatory elements such as Tregs and myeloid suppressor cells begin to operate.

This concept of signal order might also explain why there are many seemingly contradictory reports on the effects of TLR signaling and radiation responses ranging from protection, mitigation and adaptation all the way to sensitization [68–70]. Timing, dosing, location and TLR profile may justify some of these inconsistencies as will the study endpoint that was chosen, e.g. DNA damage, survival or cytokine release and which cell type carries the TLR that is being stimulated, i.e. immune cells vs epithelial cells. Several reports outside the radiation field on combining different TLR signals are hinting at the possibility that whichever signal comes first will affect the response to the respective second signal [13], ranging from cooperation [27] to antagonism [71–73]. For instance, even when separated by

days or weeks, successive acute lung injury with infection can amplify responses causing persistent systemic inflammation [27], that wouldn't be present for either signal alone, presumably through common, feed-forward DAMP- and PAMP-driven TLR signaling mechanisms that use ROS as mediators. We believe that this is a crucial point in the context of whole body irradiation, particularly when one thinks about the interaction between the gut microbiome and the irradiated host and the implications this has systemically [74].

Because TLR signaling can translate seemingly divergent signals such as radiation and PAMPS into an universal one, namely ROS, one can easily appreciate the link to non-targeted effects and carcinogenesis in this context [75–77]. Whether or not a particular combination of TLR signals leads to recurring oxidative stress and chronic genotoxicity will in part depend on the intensity and the timing of different stressors. For instance, LPS seems able to add to 4Gy-DNA damage only within a certain time frame [78]. But such carcinogenic interactions are extremely complex [79] and, ironically, they may be particularly relevant for the lower end of the dose spectrum where direct radiation damage does not dominate [80].

5. TLR in the context of whole body exposures and normal tissue damage

The gut is an extremely rich reservoir of PAMPs [81] and since it can become leaky upon radiation injury [82], it is a specific case of the interaction between radiation damage and TLRs. LPS may cross the gut barrier in whole body irradiated animals and can turn into a systemic immune adjuvant leading to radiation-induced autoimmunity [74]. The TLRs at intestinal surfaces seem as important as those on immune cells at sensing danger [83] and it is reasonable to suggest that in such a radiation-damaged gut, DAMPs may work in cohort with multiple PAMPs to engage TLR signaling cascades. Not surprisingly, the profile of commensal bacteria can determine intrinsic radiation sensitivity of the animal [84, 85], as can the profile of existing TLRs [84]. Taken further, the use of TLR agonists or antagonists is therefore a legitimate and attractive approach to modify radiation responses that has a long history [86]. A TLR5 agonist derived from flagellin for instance can protect mice and nonhuman primates from both gastrointestinal and hematological toxicity when given before 8–13Gy whole body irradiation (WBI) with the liver being the central coordinator of this response [87–89]. Driving TLR 2, 3, 4 and 9 has similar radioprotective effects [70, 90–92] and TLR 2, 4, 5 and 9 signaling can also trigger mitigation, post radiation exposure [70, 88, 92–94] all within the range of 4.5–13Gy of WBI, depending on the experimental model and the end-point investigated. Whether or not the mechanisms behind radiation protection are identical to the ones that confer mitigation is not entirely clear but chemical library screening suggests this is not necessarily the case [95, 96]. In this context, one might also think about why it is that TLR agonists are often much better radiation protectors than they are mitigators [70], highlighting again the importance of the order of DAMP and/or PAMP signal. Even if mitigation can be achieved by PAMP-TLR engagement (post radiation exposure), the timeframe within which this can be accomplished is usually much shorter (5mins–24h) or the radiation doses much lower (8.5–9Gy) than when given before radiation exposure (i.e. protection, 30mins–48h before 10–13Gy) [88, 92].

Most data on TLR engagement in this context seem to point at NF κ B induction, sending anti-apoptotic, anti-oxidant and proliferative signals that confer the survival of intestinal and hematopoietic stem cells and lead to the observed radiation protection, even if IFN gene induction may also contribute [84, 88, 91, 97]. In other words, the down-stream induction of immuno-modulatory genes that is primarily associated with TLR engagement may only play a secondary role in mediating radiation protection and mitigation. Although studies on the importance of TNFR1 in the LPS-driven radioprotection of the intestine would argue against that, as would the observed M2-polarization of macrophages during mitigation and the enhanced radiation fibrosis in TLR2/4 knockout mice [93, 98, 99].

It may seem paradoxical at first that germ-free mice, i.e. without any PAMPs are widely considered as being relatively more radioresistant [85, 100]. But the assumption is that this is largely due to a reduced rate of infection during the acute radiation syndrome and only secondary related to effects on stem cell renewal. This is of course aside from the fact that on occasion the term ‘specific pathogen-free’ may have been erroneously interchanged with ‘germ-free’. Adding yet another layer of complexity, blockade of TLR3 even in germ-free mice can protect and rescue from GI radiation syndrome indicating that not only PAMPs but also DAMPs are important, in this case mediating crypt cell death by driving the TLR3/TRIF/RIP1 axis and inducing more, not less apoptosis [37]. It has also been suggested that the TLR adapter protein MyD88 directly interferes with DNA damage repair in crypt cells, inhibiting both HR and NHEJ and hence exacerbating radiation-induced apoptosis [101]. In the brain, LPS-induced neuroinflammation present at the time of irradiation makes young mice very sensitive to the radiation damage and slows recovery, perhaps also illustrating the tissue specific nature inherent to the TLR signaling axis [68]. It is worth mentioning here that the use of antibiotics to prevent infection and/or study the role of PAMP-TLR signals is generally tricky as some of these compounds have direct radiation-modulatory effects that are independent of their antimicrobial properties [95] and ideally results should be confirmed with alternative approaches such as in vitro studies, in vivo germ-free and/or knock-out models.

6. TLRs in the context of local tumor irradiation

The concept of alerting one’s own immune system to a growing tumor through pattern recognition is not new, dating back more than a century ago to William B Coley and his use of bacterial injections into cancer patients (Coley’s toxin). Today we know that part of the therapeutic efficacy of cancer RT actually comes from activating the same innate TLR signaling pathways [31] presumably by inducing an immunogenic tumor cell death and the release of DAMPs that translate into anti-tumor immunity [56]. Sensing of cytosolic DNA and the activation of the stimulator of interferon genes (STING) pathway may be particularly important in unleashing this immune component during radiation therapy [102–104]. We and others have observed that radiation can dose-dependently drive MHC expression on tumor cells after as low as 1Gy and certainly above that in a type I IFN- and NF κ B- dependent manner and so enhance tumor antigen presentation with the chance for recognition by T cells [105–107]. Indeed, spontaneous T cell responses have already indicated that type I IFN is an essential signal that drives CD8 T cells [108, 109].

More importantly, the concept of releasing danger signals from irradiated tumor cells, and hence the activation of TLR, may become particularly pertinent in the current climate of replacing conventional fractionation with higher dose/fraction for some solid cancers. One can easily make a case for radiation dose dependency in the type, the timing and the amplitude of the danger signals that are released and/or the TLRs that are expressed (see section 3). In fact, we have reasons to believe that there may be an optimal radiation dose that translates tumor death and the release of danger signals into maximal anti-tumor immunity, namely about 7–8Gy/fraction [110, 111]. Extrapolating this to the clinic, one would assume that such moderate hypofractionation is –immunologically speaking– superior to smaller fraction size, notwithstanding complex inflammatory and vascular cascades that may also drive a different radiobiology altogether [112, 113]. Importantly, this does not mean that immune intervention shouldn't be integrated into conventional RT, when hypofractionation is not an option. Rather, the timing and perhaps the type of the immune intervention may have to be individually adjusted under such circumstances in order to maximize the therapeutic ratio.

There are currently numeral innovative clinical trials under way aiming to capitalize on pattern recognition and the power of the immune system by using TLR agonists within the context of cancer RT [114]. Most notably for solid cancers, Imiquimod (IMQ, 3M Pharmaceuticals), a nucleoside analogue of the imidazoquinoline family and a TLR7/8 agonist that was approved by the FDA in 2004 for the topical use against superficial basal cell carcinoma, extended from its original 1997 approval for other skin growths. IMQ is currently in clinical trials in combination with radiation to treat breast cancer metastasis (5x 6Gy; NCT01421017) or glioma (33x 1.8Gy; NCT01400672) (www.clinicaltrials.gov). IMQ's actions seem multifaceted, sometimes independent of TLRs, namely through redirecting the adenosine receptor-mediated immune suppression to inhibit oncogenic hedgehog signaling and by driving tumor cell death [115, 116]. A TLR4 agonist is being added to RT of sarcoma (NCT02180698) while TLR9 agonists are very promising as adjuvants to the RT of solid and hematological cancers [117, 118].

However, danger signaling in the context of tumor irradiation may not always be desirable as some of the pattern recognition receptors can send the wrong signals, TLR9, for instance, activating myeloid cells in the 13Gy-irradiated tumor microenvironment that end up supporting tumor growth in a mouse melanoma model [119]. Many TLR can also be found on tumor cells, possibly driving chronic inflammation, carcinogenesis and metastasis [7, 11, 120] and when stimulated further by agonist binding (for instance for TLR7 or 8), unwanted pro-survival and proliferative forces begin to operate and support a more radioresistant phenotype, similar to what has been observed in the normal tissue context and whole-body exposures (see above) [121].

7. Concluding remarks

One could easily argue that TLRs represent our own, individual security agency, systemically scanning for dangers from within and from outside. Radiation biologists recognize the importance of redox in responding to danger and easily appreciate how redox and TLRs crucially interface radiation damage with the immune system. Clearly, a better

understanding of the TLR signaling network in its full potency and complexity would give us a powerful tool to shape the interpretation of radiation tissue damage and turn it into normal tissue protection or anti-tumor immunity, as needed.

Acknowledgments

The authors are very grateful to Drs. Keisuke S. Iwamoto and William H. McBride for valuable scientific discussions and proofreading in preparation of this manuscript. Financial support came from the US Army MTA W81XWH-11-1-0531 and NIAID 2U19 AI067769.

References

1. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature*. 1997; 388:394–397. [PubMed: 9237759]
2. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol*. 1989; 54(Pt 1):1–13. [PubMed: 2700931]
3. Matzinger P. The danger model: a renewed sense of self. *Science*. 2002; 296:301–305. [PubMed: 11951032]
4. Medzhitov R. Approaching the asymptote: 20 years later. *Immunity*. 2009; 30:766–775. [PubMed: 19538928]
5. Curtin JF, Liu N, Candolfi M, Xiong W, Assi H, Yagiz K, Edwards MR, Michelsen KS, Kroeger KM, Liu C, Muhammad AK, Clark MC, Arditi M, Comin-Anduix B, Ribas A, Lowenstein PR, Castro MG. HMGB1 mediates endogenous TLR2 activation and brain tumor regression. *PLoS Med*. 2009; 6:e10. [PubMed: 19143470]
6. Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR, Devera ME, Liang X, Tor M, Billiar T. The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunol Rev*. 2007; 220:60–81. [PubMed: 17979840]
7. Sato Y, Goto Y, Narita N, Hoon DS. Cancer Cells Expressing Toll-like Receptors and the Tumor Microenvironment. *Cancer Microenviron*. 2009; 2(Suppl 1):205–214. [PubMed: 19685283]
8. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature*. 2003; 425:516–521. [PubMed: 14520412]
9. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity*. 2011; 34:637–650. [PubMed: 21616434]
10. Kanzler H, Barrat FJ, Hessel EM, Coffman RL. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. *Nat Med*. 2007; 13:552–559. [PubMed: 17479101]
11. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. *Nat Rev Cancer*. 2009; 9:57–63. [PubMed: 19052556]
12. O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors - redefining innate immunity. *Nat Rev Immunol*. 2013; 13:453–460. [PubMed: 23681101]
13. Piccinini AM, Midwood KS. DAMPenning inflammation by modulating TLR signalling. *Mediators Inflamm*. 2010; 2010:1–21.
14. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature*. 1998; 392:245–252. [PubMed: 9521319]
15. Gallucci S, Lolkema M, Matzinger P. Natural adjuvants: endogenous activators of dendritic cells. *Nat Med*. 1999; 5:1249–1255. [PubMed: 10545990]
16. Nair-Gupta P, Baccarini A, Tung N, Seyffer F, Florey O, Huang Y, Banerjee M, Overholtzer M, Roche PA, Tampe R, Brown BD, Amsen D, Whiteheart SW, Blander JM. TLR signals induce phagosomal MHC-I delivery from the endosomal recycling compartment to allow cross-presentation. *Cell*. 2014; 158:506–521. [PubMed: 25083866]
17. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol*. 2003; 21:685–711. [PubMed: 12615891]
18. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest*. 2012; 122:787–795. [PubMed: 22378047]

19. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun*. 2000; 68:7010–7017. [PubMed: 11083826]
20. Fitzner N, Clauberg S, Essmann F, Liebmann J, Kolb-Bachofen V. Human skin endothelial cells can express all 10 TLR genes and respond to respective ligands. *Clin Vaccine Immunol*. 2008; 15:138–146. [PubMed: 17978010]
21. Schaue D, McBride WH. Links between innate immunity and normal tissue radiobiology. *Radiat Res*. 2010; 173:406–417. [PubMed: 20334512]
22. Schaue D, Kachikwu EL, McBride WH. Cytokines in radiobiological responses: a review. *Radiat Res*. 2012; 178:505–523. [PubMed: 23106210]
23. Jones GR. Free radicals in immunological killing: the case of tumor necrotising factor (TNF). *Med Hypotheses*. 1986; 21:267–271. [PubMed: 3642198]
24. Watanabe N, Niitsu Y, Neda H, Sone H, Yamauchi N, Maeda M, Urushizaki I. Cytocidal mechanism of TNF: effects of lysosomal enzyme and hydroxyl radical inhibitors on cytotoxicity. *Immunopharmacol Immunotoxicol*. 1988; 10:109–116. [PubMed: 2834428]
25. Bubici C, Papa S, Dean K, Franzoso G. Mutual cross-talk between reactive oxygen species and nuclear factor-kappa B: molecular basis and biological significance. *Oncogene*. 2006; 25:6731–6748. [PubMed: 17072325]
26. Yang D, Elner SG, Bian ZM, Till GO, Petty HR, Elner VM. Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells. *Exp Eye Res*. 2007; 85:462–472. [PubMed: 17765224]
27. Xiang M, Fan J. Association of Toll-like receptor signaling and reactive oxygen species: a potential therapeutic target for posttrauma acute lung injury. *Mediators Inflamm*. 2010; 2010:1–10.
28. Fan J, Li Y, Levy RM, Fan JJ, Hackam DJ, Vodovotz Y, Yang H, Tracey KJ, Billiar TR, Wilson MA. Hemorrhagic shock induces NAD(P)H oxidase activation in neutrophils: role of HMGB1-TLR4 signaling. *J Immunol*. 2007; 178:6573–6580. [PubMed: 17475888]
29. Fan J, Kapus A, Marsden PA, Li YH, Oreopoulos G, Marshall JC, Frantz S, Kelly RA, Medzhitov R, Rotstein OD. Regulation of Toll-like receptor 4 expression in the lung following hemorrhagic shock and lipopolysaccharide. *J Immunol*. 2002; 168:5252–5259. [PubMed: 11994482]
30. McBride WH, Chiang CS, Olson JL, Wang CC, Hong JH, Pajonk F, Dougherty GJ, Iwamoto KS, Pervan M, Liao YP. A sense of danger from radiation. *Radiat Res*. 2004; 162:1–19. [PubMed: 15222781]
31. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, Yang H, Amigorena S, Ryffel B, Barrat FJ, Saftig P, Levi F, Lidereau R, Nogues C, Mira JP, Chompret A, Joulin V, Clavel-Chapelon F, Bourhis J, Andre F, Delaloge S, Tursz T, Kroemer G, Zitvogel L. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med*. 2007; 13:1050–1059. [PubMed: 17704786]
32. Perez CA, Fu A, Onishko H, Hallahan DE, Geng L. Radiation induces an antitumour immune response to mouse melanoma. *Int J Radiat Biol*. 2009; 85:1126–1136. [PubMed: 19995238]
33. Golden EB, Frances D, Pellicciotta I, Demaria S, Helen Barcellos-Hoff M, Formenti SC. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. *Oncoimmunology*. 2014; 3:e28518. [PubMed: 25071979]
34. Shan YX, Jin SZ, Liu XD, Liu Y, Liu SZ. Ionizing radiation stimulates secretion of pro-inflammatory cytokines: dose-response relationship, mechanisms and implications. *Radiat Environ Biophys*. 2007; 46:21–29. [PubMed: 17072632]
35. El-Saghire H, Michaux A, Thierens H, Baatout S. Low doses of ionizing radiation induce immune-stimulatory responses in isolated human primary monocytes. *Int J Mol Med*. 2013; 32:1407–1414. [PubMed: 24085242]
36. Tang D, Kang R, Zeh HJ 3rd, Lotze MT. High-mobility group box 1, oxidative stress, and disease. *Antioxidants & redox signaling*. 2011; 14:1315–1335. [PubMed: 20969478]
37. Takemura N, Kawasaki T, Kunisawa J, Sato S, Lamichhane A, Kobiyama K, Aoshi T, Ito J, Mizuguchi K, Karupuchamy T, Matsunaga K, Miyatake S, Mori N, Tsujimura T, Satoh T, Kumagai Y, Kawai T, Standley DM, Ishii KJ, Kiyono H, Akira S, Uematsu S. Blockade of TLR3

- protects mice from lethal radiation-induced gastrointestinal syndrome. *Nat Commun.* 2014; 5:3492. [PubMed: 24637670]
38. Tsukimoto M, Homma T, Ohshima Y, Kojima S. Involvement of purinergic signaling in cellular response to gamma radiation. *Radiat Res.* 2010; 173:298–309. [PubMed: 20199215]
 39. Zhang M, Zhang B, Guo Y, Zhang L, Yang S, Yin L, Vidyasagar S, Maguire D, Swarts S, Zhang Z, Zhang A, Okunieff P. Alteration of circulating mitochondrial DNA concentration after irradiation. *Adv Exp Med Biol.* 2013; 765:371–377. [PubMed: 22879058]
 40. Stangl S, Themelis G, Friedrich L, Ntziachristos V, Sarantopoulos A, Molls M, Skerra A, Multhoff G. Detection of irradiation-induced, membrane heat shock protein 70 (Hsp70) in mouse tumors using Hsp70 Fab fragment. *Radiother Oncol.* 2011; 99:313–316. [PubMed: 21704400]
 41. Rubner Y, Muth C, Strnad A, Derer A, Sieber R, Buslei R, Frey B, Fietkau R, Gaipl US. Fractionated radiotherapy is the main stimulus for the induction of cell death and of Hsp70 release of p53 mutated glioblastoma cell lines. *Radiat Oncol.* 2014; 9:89. [PubMed: 24678590]
 42. Schmid TE, Multhoff G. Radiation-induced stress proteins - the role of heat shock proteins (HSP) in anti- tumor responses. *Curr Med Chem.* 2012; 19:1765–1770. [PubMed: 22414085]
 43. Obeid M, Panaretakis T, Joza N, Tufi R, Tesniere A, van Endert P, Zitvogel L, Kroemer G. Calreticulin exposure is required for the immunogenicity of gamma-irradiation and UVC light-induced apoptosis. *Cell Death Differ.* 2007; 14:1848–1850. [PubMed: 17657249]
 44. Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, Oyabu J, Murakawa T, Nakayama H, Nishida K, Akira S, Yamamoto A, Komuro I, Otsu K. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature.* 2012; 485:251–255. [PubMed: 22535248]
 45. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature.* 2002; 416:603–607. [PubMed: 11948342]
 46. Muruve DA, Petrilli V, Zaiss AK, White LR, Clark SA, Ross PJ, Parks RJ, Tschopp J. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature.* 2008; 452:103–107. [PubMed: 18288107]
 47. Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T, Wu J, Monks BG, Fitzgerald KA, Hornung V, Latz E. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *Journal of immunology.* 2009; 183:787–791.
 48. Stoecklein VM, Osuka A, Ishikawa S, Lederer MR, Wanke-Jellinek L, Lederer JA. Radiation exposure induces inflammasome pathway activation in immune cells. *J Immunol.* 2015; 194:1178–1189. [PubMed: 25539818]
 49. Chiang CS, Hong JH, Stalder A, Sun JR, Withers HR, McBride WH. Delayed molecular responses to brain irradiation. *International journal of radiation biology.* 1997; 72:45–53. [PubMed: 9246193]
 50. Chiang CS, Liu WC, Jung SM, Chen FH, Wu CR, McBride WH, Lee CC, Hong JH. Compartmental responses after thoracic irradiation of mice: strain differences. *International journal of radiation oncology, biology, physics.* 2005; 62:862–871.
 51. Ohshima Y, Tsukimoto M, Takenouchi T, Harada H, Suzuki A, Sato M, Kitani H, Kojima S. gamma-Irradiation induces P2X(7) receptor-dependent ATP release from B16 melanoma cells. *Biochim Biophys Acta.* 2010; 1800:40–46. [PubMed: 19854240]
 52. Nishimaki N, Tsukimoto M, Kitami A, Kojima S. Autocrine regulation of gamma-irradiation-induced DNA damage response via extracellular nucleotides-mediated activation of P2Y6 and P2Y12 receptors. *DNA Repair (Amst).* 2012; 11:657–665. [PubMed: 22682873]
 53. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature.* 2010; 464:104–107. [PubMed: 20203610]
 54. Miller YI, Choi SH, Wiesner P, Fang L, Harkewicz R, Hartvigsen K, Boullier A, Gonen A, Diehl CJ, Que X, Montano E, Shaw PX, Tsimikas S, Binder CJ, Witztum JL. Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. *Circulation research.* 2011; 108:235–248. [PubMed: 21252151]

55. Kazama H, Ricci JE, Herndon JM, Hoppe G, Green DR, Ferguson TA. Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. *Immunity*. 2008; 29:21–32. [PubMed: 18631454]
56. Inoue H, Tani K. Multimodal immunogenic cancer cell death as a consequence of anticancer cytotoxic treatments. *Cell Death Differ*. 2014; 21:39–49. [PubMed: 23832118]
57. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature*. 2005; 436:1186–1190. [PubMed: 15995699]
58. Kim KW, Kim SH, Shin JG, Kim GS, Son YO, Park SW, Kwon BH, Kim DW, Lee CH, Sol MY, Jeong MH, Chung BS, Kang CD. Direct injection of immature dendritic cells into irradiated tumor induces efficient antitumor immunity. *Int J Cancer*. 2004; 109:685–690. [PubMed: 14999775]
59. Menendez D, Shatz M, Azzam K, Garantziotis S, Fessler MB, Resnick MA. The Toll-like receptor gene family is integrated into human DNA damage and p53 networks. *PLoS Genet*. 2011; 7:e1001360. [PubMed: 21483755]
60. Taura M, Eguma A, Suico MA, Shuto T, Koga T, Komatsu K, Komune T, Sato T, Saya H, Li JD, Kai H. p53 regulates Toll-like receptor 3 expression and function in human epithelial cell lines. *Mol Cell Biol*. 2008; 28:6557–6567. [PubMed: 18779317]
61. Ermolaeva MA, Segref A, Dakhovnik A, Ou HL, Schneider JI, Utermohlen O, Hoppe T, Schumacher B. DNA damage in germ cells induces an innate immune response that triggers systemic stress resistance. *Nature*. 2013; 501:416–420. [PubMed: 23975097]
62. Chovatiya R, Medzhitov R. Honor thy Go(na)ds. *Immunol Cell Biol*. 2013; 91:597–598. [PubMed: 24060964]
63. Schaeue D, Micewicz ED, Ratikan JA, Xie MW, Cheng G, McBride WH. Radiation and Inflammation. *Semin Radiat Oncol*. 2015; 25:4–10. [PubMed: 25481260]
64. McDonald JT, Kim K, Norris AJ, Vlasi E, Phillips TM, Lagadec C, Della Donna L, Ratikan J, Szelag H, Hlatky L, McBride WH. Ionizing radiation activates the Nrf2 antioxidant response. *Cancer Res*. 2010; 70:8886–8895. [PubMed: 20940400]
65. Smith JT, Willey NJ, Hancock JT. Low dose ionizing radiation produces too few reactive oxygen species to directly affect antioxidant concentrations in cells. *Biol Lett*. 2012; 8:594–597. [PubMed: 22496076]
66. Hong JH, Chiang CS, Campbell IL, Sun JR, Withers HR, McBride WH. Induction of acute phase gene expression by brain irradiation. *Int J Radiat Oncol Biol Phys*. 1995; 33:619–626. [PubMed: 7558951]
67. Rodel F, Frey B, Gaipf U, Keilholz L, Fournier C, Manda K, Schollnberger H, Hildebrandt G, Rodel C. Modulation of inflammatory immune reactions by low-dose ionizing radiation: molecular mechanisms and clinical application. *Curr Med Chem*. 2012; 19:1741–1750. [PubMed: 22414082]
68. Kalm M, Roughton K, Blomgren K. Lipopolysaccharide sensitized male and female juvenile brains to ionizing radiation. *Cell Death Dis*. 2013; 4:e962. [PubMed: 24336082]
69. Chen H, Wang ZD, Chen MS, Zhang XQ, Shen LP, Zhang JX, Chen Y. Activation of Toll-like receptors by intestinal microflora reduces radiation-induced DNA damage in mice. *Mutat Res Genet Toxicol Environ Mutagen*. 2014; 774:22–28. [PubMed: 25440907]
70. Saha S, Bhanja P, Liu L, Alfieri AA, Yu D, Kandimalla ER, Agrawal S, Guha C. TLR9 agonist protects mice from radiation-induced gastrointestinal syndrome. *PLoS One*. 2012; 7:e29357. [PubMed: 22238604]
71. Ando K, Hasegawa K, Shindo K, Furusawa T, Fujino T, Kikugawa K, Nakano H, Takeuchi O, Akira S, Akiyama T, Gohda J, Inoue J, Hayakawa M. Human lactoferrin activates NF-kappaB through the Toll-like receptor 4 pathway while it interferes with the lipopolysaccharide-stimulated TLR4 signaling. *FEBS J*. 2010; 277:2051–2066. [PubMed: 20345905]
72. Puddu P, Latorre D, Carollo M, Catizone A, Ricci G, Valenti P, Gessani S. Bovine lactoferrin counteracts Toll-like receptor mediated activation signals in antigen presenting cells. *PLoS One*. 2011; 6:e22504. [PubMed: 21799877]
73. Zheng Y, Qin Z, Ye Q, Chen P, Wang Z, Yan Q, Luo Z, Liu X, Zhou Y, Xiong W, Ma J, Li G. Lactoferrin suppresses the Epstein-Barr virus-induced inflammatory response by interfering with pattern recognition of TLR2 and TLR9. *Lab Invest*. 2014; 94:1188–1199. [PubMed: 25068657]

74. Paulos CM, Wrzesinski C, Kaiser A, Hinrichs CS, Chieppa M, Cassard L, Palmer DC, Boni A, Muranski P, Yu Z, Gattinoni L, Antony PA, Rosenberg SA, Restifo NP. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8⁺ T cells via TLR4 signaling. *J Clin Invest*. 2007; 117:2197–2204. [PubMed: 17657310]
75. Lorimore SA, Coates PJ, Scobie GE, Milne G, Wright EG. Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects? *Oncogene*. 2001; 20:7085–7095. [PubMed: 11704832]
76. Ochi A, Graffeo CS, Zambirinis CP, Rehman A, Hackman M, Fallon N, Barilla RM, Henning JR, Jamal M, Rao R, Greco S, Deutsch M, Medina-Zea MV, Bin Saeed U, Ego-Osuala MO, Hajdu C, Miller G. Toll-like receptor 7 regulates pancreatic carcinogenesis in mice and humans. *J Clin Invest*. 2012; 122:4118–4129. [PubMed: 23023703]
77. Salcedo R, Cataisson C, Hasan U, Yuspa SH, Trinchieri G. MyD88 and its divergent toll in carcinogenesis. *Trends Immunol*. 2013; 34:379–389. [PubMed: 23660392]
78. McAllister KA, Lorimore SA, Wright EG, Coates PJ. In vivo interactions between ionizing radiation, inflammation and chemical carcinogens identified by increased DNA damage responses. *Radiat Res*. 2012; 177:584–593. [PubMed: 22463680]
79. Trott KR, Rosemann M. Molecular mechanisms of radiation carcinogenesis and the linear, non-threshold dose response model of radiation risk estimation. *Radiat Environ Biophys*. 2000; 39:79–87. [PubMed: 10929376]
80. Morgan WF, Sowa MB. Non-targeted effects induced by ionizing radiation: mechanisms and potential impact on radiation induced health effects. *Cancer Lett*. 2013; 356:17–21. [PubMed: 24041870]
81. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature*. 2012; 489:220–230. [PubMed: 22972295]
82. Nejdfors P, Ekelund M, Westrom BR, Willen R, Jeppsson B. Intestinal permeability in humans is increased after radiation therapy. *Dis Colon Rectum*. 2000; 43:1582–1587. discussion 1587–1588. [PubMed: 11089597]
83. Santaolalla R, Abreu MT. Innate immunity in the small intestine. *Current opinion in gastroenterology*. 2012; 28:124–129. [PubMed: 22241076]
84. Liu C, Zhang C, Mitchel RE, Cui J, Lin J, Yang Y, Liu X, Cai J. A critical role of toll-like receptor 4 (TLR4) and its' in vivo ligands in basal radio-resistance. *Cell Death Dis*. 2013; 4:e649. [PubMed: 23722538]
85. Crawford PA, Gordon JI. Microbial regulation of intestinal radiosensitivity. *Proc Natl Acad Sci U S A*. 2005; 102:13254–13259. [PubMed: 16129828]
86. Smith WW, Alderman IM, Gillespie RE. Increased survival in irradiated animals treated with bacterial endotoxins. *Am J Physiol*. 1957; 191:124–130. [PubMed: 13478698]
87. Vijay-Kumar M, Aitken JD, Sanders CJ, Frias A, Sloane VM, Xu J, Neish AS, Rojas M, Gewirtz AT. Flagellin treatment protects against chemicals, bacteria, viruses, and radiation. *J Immunol*. 2008; 180:8280–8285. [PubMed: 18523294]
88. Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, Kurnasov OV, Fort FL, Osterman AL, Didonato JA, Feinstein E, Gudkov AV. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science*. 2008; 320:226–230. [PubMed: 18403709]
89. Burdelya LG, Brackett CM, Kojouharov B, Gitlin II, Leonova KI, Gleiberman AS, Aygun-Sunar S, Veith J, Johnson C, Haderski GJ, Stanhope-Baker P, Allamaneni S, Skitzki J, Zeng M, Martsen E, Medvedev A, Scheblyakov D, Artemicheva NM, Logunov DY, Gintsburg AL, Naroditsky BS, Makarov SS, Gudkov AV. Central role of liver in anticancer and radioprotective activities of Toll-like receptor 5 agonist. *Proc Natl Acad Sci U S A*. 2013; 110:E1857–1866. [PubMed: 23630282]
90. Ciorba MA, Riehl TE, Rao MS, Moon C, Ee X, Nava GM, Walker MR, Marinshaw JM, Stappenbeck TS, Stenson WF. Lactobacillus probiotic protects intestinal epithelium from radiation injury in a TLR-2/cyclo-oxygenase-2-dependent manner. *Gut*. 2012; 61:829–838. [PubMed: 22027478]
91. Talas M, Szolgay E. Radioprotective activity of interferon inducers. *Arch Virol*. 1978; 56:309–315. [PubMed: 348157]

92. Shakhov AN, Singh VK, Bone F, Cheney A, Kononov Y, Krasnov P, Bratanova-Toshkova TK, Shakhova VV, Young J, Weil MM, Panoskaltsis-Mortari A, Orschell CM, Baker PS, Gudkov A, Feinstein E. Prevention and mitigation of acute radiation syndrome in mice by synthetic lipopeptide agonists of Toll-like receptor 2 (TLR2). *PLoS One*. 2012; 7:e33044. [PubMed: 22479357]
93. Lacave-Lapalun JV, Benderitter M, Linard C. Flagellin or lipopolysaccharide treatment modified macrophage populations after colorectal radiation of rats. *J Pharmacol Exp Ther*. 2013; 346:75–85. [PubMed: 23596059]
94. Zhang C, Lin J, Cui J, Li B, Liu C, Wang J, Gao F, Cai J. Radioprotection of bone marrow hematopoiesis by CpG-oligodeoxynucleotides administered to mice after total-body irradiation. *J Radiat Res*. 2011; 52:828–833. [PubMed: 22104272]
95. Kim K, Pollard JM, Norris AJ, McDonald JT, Sun Y, Micewicz E, Pettijohn K, Damoiseaux R, Iwamoto KS, Sayre JW, Price BD, Gatti RA, McBride WH. High-throughput screening identifies two classes of antibiotics as radioprotectors: tetracyclines and fluoroquinolones. *Clin Cancer Res*. 2009; 15:7238–7245. [PubMed: 19920105]
96. Kim K, Damoiseaux R, Norris AJ, Rivina L, Bradley K, Jung ME, Gatti RA, Schiestl RH, McBride WH. High throughput screening of small molecule libraries for modifiers of radiation responses. *Int J Radiat Biol*. 2011; 87:839–845. [PubMed: 21401317]
97. Egan LJ, Eckmann L, Greten FR, Chae S, Li ZW, Myhre GM, Robine S, Karin M, Kagnoff MF. IkappaB-kinasebeta-dependent NF-kappaB activation provides radioprotection to the intestinal epithelium. *Proc Natl Acad Sci U S A*. 2004; 101:2452–2457. [PubMed: 14983030]
98. Riehl TE, Newberry RD, Lorenz RG, Stenson WF. TNFR1 mediates the radioprotective effects of lipopolysaccharide in the mouse intestine. *Am J Physiol Gastrointest Liver Physiol*. 2004; 286:G166–173. [PubMed: 14525729]
99. Paun A, Fox J, Balloy V, Chignard M, Qureshi ST, Haston CK. Combined Tlr2 and Tlr4 deficiency increases radiation-induced pulmonary fibrosis in mice. *Int J Radiat Oncol Biol Phys*. 2010; 77:1198–1205. [PubMed: 20610040]
100. McLaughlin MM, Dacquisto MP, Jacobus DP, Horowitz RE. Effects of the Germfree State on Responses of Mice to Whole-Body Irradiation. *Radiat Res*. 1964; 23:333–349. [PubMed: 14229117]
101. Lai XY, Egan LJ. Suppression of radiation-induced DNA double-strand break repair by MyD88 is accompanied by apoptosis and crypt loss in mouse colon. *Oncogenesis*. 2013; 2:e62. [PubMed: 23939014]
102. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MY, Duggan R, Wang Y, Barber GN, Fitzgerald KA, Alegre ML, Gajewski TF. STING-Dependent Cytosolic DNA Sensing Mediates Innate Immune Recognition of Immunogenic Tumors. *Immunity*. 2014; 41:830–842. [PubMed: 25517615]
103. Deng L, Liang H, Xu M, Yang X, Burnette B, Arina A, Li XD, Mauceri H, Beckett M, Darga T, Huang X, Gajewski TF, Chen ZJ, Fu YX, Weichselbaum RR. STING-Dependent Cytosolic DNA Sensing Promotes Radiation-Induced Type I Interferon-Dependent Antitumor Immunity in Immunogenic Tumors. *Immunity*. 2014; 41:843–852. [PubMed: 25517616]
104. Klarquist J, Hennies CM, Lehn MA, Reboulet RA, Feau S, Janssen EM. STING-Mediated DNA Sensing Promotes Antitumor and Autoimmune Responses to Dying Cells. *J Immunol*. 2014; 193:6124–6134. [PubMed: 25385820]
105. Wan S, Pestka S, Jubin RG, Lyu YL, Tsai YC, Liu LF. Chemotherapeutics and radiation stimulate MHC class I expression through elevated interferon-beta signaling in breast cancer cells. *PLoS One*. 2012; 7:e32542. [PubMed: 22396773]
106. Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, Camphausen K, Luiten RM, de Ru AH, Neijssen J, Griekspoor A, Mesman E, Verreck FA, Spits H, Schlom J, van Veelen P, Neeffjes JJ. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*. 2006; 203:1259–1271. [PubMed: 16636135]
107. Johnson DR, Pober JS. HLA class I heavy-chain gene promoter elements mediating synergy between tumor necrosis factor and interferons. *Mol Cell Biol*. 1994; 14:1322–1332. [PubMed: 8289810]

108. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, Lee H, Arthur CD, White JM, Kalinke U, Murphy KM, Schreiber RD. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. *J Exp Med.* 2011; 208:1989–2003. [PubMed: 21930769]
109. Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, Gajewski TF. Host type I IFN signals are required for antitumor CD8⁺ T cell responses through CD8 α ⁺ dendritic cells. *J Exp Med.* 2011; 208:2005–2016. [PubMed: 21930765]
110. Schaue D, Ratikan JA, Iwamoto KS, McBride WH. Maximizing Tumor Immunity with Fractionated Radiation. *Int J Radiat Oncol Biol Phys.* 2012; 83:1306–1310. [PubMed: 22208977]
111. Dewan MZ, Galloway AE, Kawashima N, Dewyngaert JK, Babb JS, Formenti SC, Demaria S. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res.* 2009; 15:5379–5388. [PubMed: 19706802]
112. McBride, WH.; Schaue, D. Radiation Biology of SBRT: Is There a New Biology Involved?. In: PA; AMM, editors. Hypofractionation: Scientific Concepts and Clinical Experiences. LumiText Publishing; Elliot City: 2011. p. 3-18.
113. Finkelstein SE, Timmerman R, McBride WH, Schaue D, Hoffe SE, Mantz CA, Wilson GD. The confluence of stereotactic ablative radiotherapy and tumor immunology. *Clin Dev Immunol.* 2011; 2011:439752. [PubMed: 22162711]
114. Marabelle A, Filatenkov A, Sagiv-Barfi I, Kohrt H. Radiotherapy and Toll-Like Receptor Agonists. *Semin Radiat Oncol.* 2015; 25:34–39. [PubMed: 25481264]
115. Schon MP, Schon M. Imiquimod: mode of action. *Br J Dermatol.* 2007; 157(Suppl 2):8–13. [PubMed: 18067624]
116. Wolff F, Loipetzberger A, Gruber W, Esterbauer H, Aberger F, Frischauf AM. Imiquimod directly inhibits Hedgehog signalling by stimulating adenosine receptor/protein kinase A-mediated G1I phosphorylation. *Oncogene.* 2013; 32:5574–5581. [PubMed: 23995793]
117. Milas L, Mason KA, Ariga H, Hunter N, Neal R, Valdecanas D, Krieg AM, Whisnant JK. CpG oligodeoxynucleotide enhances tumor response to radiation. *Cancer Res.* 2004; 64:5074–5077. [PubMed: 15289307]
118. Brody JD, Ai WZ, Czerwinski DK, Torchia JA, Levy M, Advani RH, Kim YH, Hoppe RT, Knox SJ, Shin LK, Wapnir I, Tibshirani RJ, Levy R. In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study. *J Clin Oncol.* 2010; 28:4324–4332. [PubMed: 20697067]
119. Gao C, Kozłowska A, Nechaev S, Li H, Zhang Q, Hossain DM, Kowolik CM, Chu P, Swiderski P, Diamond DJ, Pal SK, Raubitschek A, Kortylewski M. TLR9 signaling in the tumor microenvironment initiates cancer recurrence after radiotherapy. *Cancer Res.* 2013; 73:7211–7221. [PubMed: 24154870]
120. Huang B, Zhao J, Unkeless JC, Feng ZH, Xiong H. TLR signaling by tumor and immune cells: a double-edged sword. *Oncogene.* 2008; 27:218–224. [PubMed: 18176603]
121. Cherfils-Vicini J, Platonova S, Gillard M, Laurans L, Validire P, Caliendo R, Magdeleinat P, Mami-Chouaib F, Dieu-Nosjean MC, Fridman WH, Damotte D, Sautes-Fridman C, Cremer I. Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. *J Clin Invest.* 2010; 120:1285–1297. [PubMed: 20237413]
122. Hehrlein C, Kaiser S, Riessen R, Metz J, Fritz P, Kubler W. External beam radiation after stent implantation increases neointimal hyperplasia by augmenting smooth muscle cell proliferation and extracellular matrix accumulation. *J Am Coll Cardiol.* 1999; 34:561–566. [PubMed: 10440173]
123. Chang CT, Lin H, Ho TY, Li CC, Lo HY, Wu SL, Huang YF, Liang JA, Hsiang CY. Comprehensive assessment of host responses to ionizing radiation by nuclear factor-kappaB bioluminescence imaging-guided transcriptomic analysis. *PLoS One.* 2011; 6:e23682. [PubMed: 21887294]
124. Iwakawa M, Noda S, Ohta T, Oohira C, Tanaka H, Tsuji A, Ishikawa A, Imai T. Strain dependent differences in a histological study of CD44 and collagen fibers with an expression analysis of

- inflammatory response-related genes in irradiated murine lung. *J Radiat Res.* 2004; 45:423–433. [PubMed: 15613788]
125. Goltry KL, Epperly MW, Greenberger JS. Induction of serum amyloid A inflammatory response genes in irradiated bone marrow cells. *Radiat Res.* 1998; 149:570–578. [PubMed: 9611095]
126. Feurgard C, Boehler N, Ferezou J, Serougne C, Aigueperse J, Gourmelon P, Lutton C, Mathe D. Ionizing radiation alters hepatic cholesterol metabolism and plasma lipoproteins in Syrian hamster. *Int J Radiat Biol.* 1999; 75:757–766. [PubMed: 10405006]
127. Lee YS, Sohn KC, Jang S, Lee Y, Hwang C, Kim KH, Cho MJ, Kim CD, Lee JH. Anti-apoptotic role of S100A8 in X-ray irradiated keratinocytes. *J Dermatol Sci.* 2008; 51:11–18. [PubMed: 18325741]
128. Chaudhry MA. Radiation-induced gene expression profile of human cells deficient in 8-hydroxy-2'-deoxyguanine glycosylase. *Int J Cancer.* 2006; 118:633–642. [PubMed: 16106417]
129. Gordeeva KV. On the relation between fibrinogenase activity and raised fibrinogen concentrations in dogs during radiation sickness. *Biull Eksp Biol Med.* 1962; 54:39–42. [PubMed: 13949173]
130. Magic Z, Matic-Ivanovic S, Savic J, Poznanovic G. Ionizing radiation-induced expression of the genes associated with the acute response to injury in the rat. *Radiat Res.* 1995; 143:187–193. [PubMed: 7543208]
131. Nylund R, Lemola E, Hartwig S, Lehr S, Acheva A, Jahns J, Hildebrandt G, Lindholm C. Profiling of low molecular weight proteins in plasma from locally irradiated individuals. *J Radiat Res.* 2014; 55:674–682. [PubMed: 24570173]
132. Riekkki R, Jukkola A, Oikarinen A, Kalliainen M. Radiation therapy induces tenascin expression and angiogenesis in human skin. *Acta Derm Venereol.* 2001; 81:329–333. [PubMed: 11800138]
133. Geffrotin C, Tricaud Y, Crechet F, Castelli M, Lefaix JL, Vaiman M. Unlike tenascin-X, tenascin-C is highly up-regulated in pig cutaneous and underlying muscle tissue developing fibrosis after necrosis induced by very high-dose gamma radiation. *Radiat Res.* 1998; 149:472–481. [PubMed: 9588358]
134. Wehrhan F, Rodel F, Grabenbauer GG, Amann K, Bruckl W, Schultze-Mosgau S. Transforming growth factor beta 1 dependent regulation of Tenascin-C in radiation impaired wound healing. *Radiother Oncol.* 2004; 72:297–303. [PubMed: 15450728]

Highlights

- Toll-like receptors recognize universal molecular patterns on pathogens (i.e. ‘non-self’) and translate this as danger to alert the immune system by driving a pro-oxidant, pro-inflammatory response.
- Radiation-damaged cells experience changes in their internal redox status and send danger signals (‘damaged self’) to Toll-like receptors that interface the radiation damage with the immune system.
- External (‘non-self’) agonists of Toll-like receptors may reinforce the same danger signaling pathways that are engaged by radiation-induced ‘damaged self’ as they roll on the same internal redox rheostat and so amplify a pro-oxidant/pro-inflammatory signal that will help to alert the immune system, even when the original radiation dose is low.
- Therefore, Toll-like receptors act as sensors and amplifiers of radiation damage and can shape the interpretation of radiation tissue damage into normal tissue protection, sensitization or activation of anti-tumor immunity, respectively.

Figure 1. Toll-like receptors as sensors and amplifiers of radiation damage

Ionizing radiation and the associated damage leads to a rise in ROS and moves the internal redox rheostat towards a harmful pro-oxidant, pro-inflammatory position. If this goes above a critical threshold the cellular anti-oxidant defense machinery is engaged under the control of Keap1/Nrf-2 and HSF-1/HSPs to counter the danger of excessive free radicals. The range of available anti-oxidant tools includes GST/GSH, GCLC/GCLM, HO-1/biliverdin, UGT, NQO1, thioredoxin, peroxiredoxin, sulfiredoxin, ferritin and MnSOD. However, if the radiation dose is limited, the damage and/or ROS levels may remain below the cellular radar and fail to activate appropriate defense mechanisms. Under such circumstances, external TLR agonists such as LPS (PAMPs) may reinforce the same danger signaling pathways that would normally be engaged by radiation-induced TLR agonists (DAMPs) and so amplify a pro-oxidant/pro-inflammatory signal that will help to alert cells. Abbreviations: Reactive Oxygen Species (ROS); Kelch-like ECH-associated protein 1 (Keap1); NF-E2-related factor 2 (Nrf-2), Heat shock factor 1 (HSF-1); Heat shock proteins (HSPs); Glutathione transferase (GST); Glutathione (GSH); Glutamate cysteine ligase (GCLC); Glutamate-cysteine ligase modifier subunit (GCLM); Heme oxygenase 1 (HO-1); UDP-glucuronosyltransferase (UGT); NAD(P)H:quinone oxidoreductase 1 (NQO-1) Manganese superoxide dismutase (MnSOD).

Table 1

Toll-like receptors and their ligands

TLR	exogenous ligands (PAMPs)	endogenous ligands (DAMPs)	radiation-induced TLRs and DAMPs
TLR2 (heterodimer with TLR1 or TLR6)	bacterial lipoproteins and lipotechoic acid, fungal zymosan, viral envelope proteins	HMGB1, β -defensin-3, HSP60, HSP70, HSP gp96, biglycan, versican, serum amyloid A, hyaluronan fragments, uric acid crystals, surfactant protein A, antiphospholipid antibodies, saturated fatty acids	TLR2 expression [59] HMGB1 release in TLR2 model [5] biglycan [122] serum amyloid A [123] hyaluronan [124]
TLR3	dsRNA (viral), Poly I:C (synthetic)	mRNA	TLR3 expression [59] RNA release in TLR3 model[37]
TLR4	LPS, fungal mannan, phospholipids, viral envelope proteins	HMGB1, β -defensin-2, HSP22, HSP60, HSP70, HSP gp96, biglycan, saturated fatty acids, oxidized LDL and other oxidation products, serum amyloid A (SAA), S100A8 and S100A9 proteins, surfactant protein A, α A-crystallin, lactoferrin, uromodulin, hyaluronan and hyaluronan fragments, fibronectin and fibrinogen, heparan sulfate, uric acid crystals, tenascin-C, antiphospholipid antibodies	TLR4 expression [34, 35, 59] HMGB1 release [31, 35] HSP70 [40] serum amyloid A [123, 125] biglycan [122] LDL [126] S100A8 [127] hyaluronan [124] α A-crystallin [128] fibrinogen [129–131] tenascin-C [132–134]
TLR5	flagellin (<i>Helicobacter pylori</i> , <i>Salmonella</i>)		TLR5 expression [59]
TLR7 (with TLR8)	ssRNA (viral)	ssRNA (immune complexes), antiphospholipid antibodies, adenosine and guanosine derivatives	TLR7/8 expression [59]
TLR9	unmethylated CpG DNA (bacterial/viral)	mitochondrial DNA, DNA-IgG complexes, HMGB1, chromatin-Ig complexes	TLR9 expression [35, 59] HMGB1 release [35] mitochondrial DNA [39]
TLR10			TLR10 expression [59]
mTLR11* (with TLR12) mTLR13*	protozoan profilin-like molecules (<i>Toxoplasma gondii</i>), uropathogens, bacterial rRNA		

*
in the murine system only