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Host-Intrinsic Interferon Status in Infection and Immunity

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Abstract

Most genetic ablations of Interferon (IFN) signaling abolish both the experimentally induced IFN response and constitutive IFN, whose effects are well established in autoimmunity but understudied during infection. In host-pathogen interactions, most IFN-mediated responses are attributed to infection-driven IFN. However, IFNs confer their activity by regulating networks of Interferon Stimulated Genes (ISGs), a process that requires *de novo* transcription and translation of both IFN and downstream ISGs through feedback of IFN receptor signaling. Due to the temporal requirement for IFN activity, many rapid anti-microbial responses may instead result from pre-established IFN signature stemming from host-intrinsic processes. Addressing the permeating effects of constitutive IFN is therefore needed to accurately describe immunity as host-intrinsic or pathogen-induced.

Constitutive Interferon Permeates Deeply

Type I Interferons (IFNs) are well known for their rapid induction spike and vast amplification effects by and toward pathogenic stimuli. Recently, two necrotic cell death mechanism, necroptosis and pyroptosis, have surfaced to require IFN. Induction of IFN is also observed under certain circumstances during cell death, resulting in models that posit a stimulus-dependent IFN production pathway as a mechanism driving cell death[1–3]. Careful examination of the IFN response during cell death has led us to a curious realization: depending on the kinetics of cell death, *de novo* IFN may not impact the fate of the IFN-producing cells [4, 5]. Our results are corroborated by the vast number of published experiments using macrophages pre-activated with IFN [6–10], showing the importance of the IFN status of the cell prior to encountering a death-inducing stimulus that had set the stage for the cell death response.

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Constitutive low levels of IFN receptor (IFNAR) signaling have long been observed in humans and in mice, although understudied [11, 12]. These low levels of constitutive IFN are thought to sustain the steady-state abundance of key intermediates of the **IFNAR signaling pathway**, including STAT1 (Signal Transducer and Activator of Transcription), STAT2, IRF9 (Interferon Regulatory Factor), in order to elicit a rapid transcriptional response to infection [13] (Key Figure). Current mouse models with genetic deletion of genes involved in IFN induction and signaling - such as *Ifnar*^{-/-}, *Ifnb*^{-/-}, *Stat1*^{-/-} - abolish both the experimentally-driven IFN response as well as the self-sustaining IFN signal that relies on the same machinery for its propagation and regulation. Thus, the use of these models does not distinguish between the contributions of induced IFN from that of constitutive IFN (Box 1). Our experience [4, 5] indicates that many of the rapid responses currently attributed to pathogen-induced IFN, may instead result from the cell intrinsic IFN status. We therefore need to re-evaluate our understanding of how IFNs function to distinguish the contribution of host-intrinsic IFN signature with that of pathogen-induced IFN response. Here we review the potential sources of constitutive IFN, including intrinsic signals – such as DNA damage and stress responses - as well as microbial stimuli. These data highlight the role and importance of the constitutive/tonic IFN in sustaining the expression of **Interferon Stimulated Genes (ISGs)** as the effectors of many biological activities ascribed to *de novo* IFN signaling.

IFNs Act by Modulating Global Immune Status

Interferons were initially named after a phenomenon in which infection with a first virus “interferes” with the establishment of a subsequent virus in primates and humans [14]. It later became apparent that the proteins responsible for the host-acquired resistance to secondary infection - the Interferons (IFNs) - did not “attack” viruses directly. Instead, IFNs act by transcriptional upregulation of large networks of genes that either have direct anti-microbial properties or are able to mobilize a global host immune response across different species [13, 15, 16]. Furthermore, as global immune modulators, inappropriate IFN responses can drive pathology in certain infections such as *Listeria monocytogenes* and *Mycobacterium tuberculosis* [17, 18].

Type I IFNs exert their immune-modulatory functions by binding to dimeric cell surface receptors, thereby activating signal transduction pathways through STATs and IRFs to upregulate networks of genes collectively known as **Interferon Stimulated Genes (ISGs)** or Interferon Regulated Genes (IRGs) [19] (Key Figure, Figure 1). Many infections drive the transcription of *Ifn* genes. However, despite the multitude of pathways that can result in *Ifn* transcription during infection, IFN proteins are not always made and their function can be blocked [20, 21]. Perhaps most strikingly, depending on the timing in which a physiological output is observed, infection-driven IFN may not have time to elicit feedback-dependent signals to mediate the observed phenotype. In the next section, we will discuss the timing of type I IFN response and an infection model of *Legionella pneumophila* in which transcription and translation are decoupled.

The Timing and Synthesis of an Infection-Driven Interferon Response

The very first studies on the “Interference phenomenon” had found that at least 4 hours of incubation at 37°C with the first virus were required for interference to develop against a subsequent virus in the chorio-allantoic membrane of the chick embryo[22]. The time window of at least 4 hours is consistent with *de novo* induction of transcriptional and translational IFN- responses to attain functional “activation” of a cell population. What is the cell population experiencing during these early hours of viral pathogen encounter?

Upon encountering a microbe, different pathogen pattern recognition receptors (PRRs) including **TLRs** and **nucleic acid sensors** across mammalian species, undergo different signaling kinetics. For instance, double-stranded RNA and bacterial lipopolysaccharide (LPS) trigger TLR3 and TLR4, respectively, to activate the TRIF/IRF3 pathway to rapidly transcribe *Ifn* genes. IRF3 activation peaks within 1-2 hours downstream of TLR3 and TLR4 stimulation[23] in murine macrophages, with robust STAT1 phosphorylation downstream of IFNAR signaling also observed by 2 hours[24], attesting to the high rate of IFN β protein synthesis downstream of TLR stimulation [25]. In contrast, cytosolic nucleic acid sensing via cGAS (DNA-sensing) or RIG-I (RNA-sensing) pathways require overnight treatment of macrophages or fibroblasts to engage IFNAR signaling [26]. In mouse and human macrophages, STING or RIG-I mediated transcription of *Ifn* genes require 3-4 hours [27] with STAT1 phosphorylation downstream of IFNAR feedback starting at 3-4 hours and peaking at 5-6 hours[28]. It has been well established that STING driven induction of *Ifn* gene is driven by IRF3/IRF3 homodimers and p50/p65 heterodimers [29]. In select cell types, such as CD8 T cells, IRF2 is necessary to attenuate hyper-induction of *Ifn* genes to maintain cellular homeostasis [30]. In resting cells, constitutive IFN thus appears to be maintained by a balance of IRF2 and IRF3 activity, since IRF7 itself is an ISG.

Downstream of IFNAR activation, a substantial amount of time is again required for the synthesis of ISG-encoded proteins. Indeed, recent findings across 11 primary mouse immune cells and 900 genes showed an average transcriptional peak of 2 hours post IFN α 3 stimulation [13]. Besides coding genes, there could be as many non-coding RNAs (lncRNAs) to negatively regulate the strength and duration of IFN-signaling [31]. The translational timeframe is more difficult to measure, since protein translation efficiency hinges on the combinatorial effects of translation initiation control, tRNA availability, and codon frequency[32–34]. Additionally, protein complex stability and function can depend on the availability of individual sub-units[35]. Assuming a simple model in which transcription of ISGs is immediately followed by their translation and activity, a complete cycle of *de novo* IFN response from PRR-recognition of pathogens to synthesis of anti-microbial ISGs would require on the order of 4-8 hours after the initial stimulation event [26]. Traditional *in vitro* methods of macrophage polarization allow cells to respond to IFN γ or other IFN-eliciting stimuli including LPS for 16-24 hours to establish an activated phenotype. Therefore, a successful infection-driven IFN response requires time to be established, and an IFN-dependent anti-microbial response that can be readily observed within the first 4 hours of pathogen encounter, such as some cases of pyroptosis [36–38] and necroptosis [39] is unlikely to be driven by *de novo* synthesized IFN based on the timeline of ISGs synthesis, which requires a much longer time than the observed cell death response.

While it may seem obvious, a successful IFN response also requires synthesis of the cytokine. A puzzling case lies within the macrophage pyroptosis response toward *Legionella pneumophila*. In mice, caspase-11 dependent pyroptosis toward vacuole-resident bacteria, such as *L. pneumophila*, is thought to be potentiated by infection-driven IFN [11,12]. During *L. pneumophila* challenge in mice, bacterial DNA/RNA triggers a robust *Ifnb* transcriptional response that is mediated by the cGAS/STING and RIG1/MAVS pathways [40, 41]. However, this event is unaccompanied by an accumulation of IFN β protein to match the transcriptional spike [42]. Furthermore, *L. pneumophila* translocates into the host cell effectors capable of blocking host cell protein translation, where only a select number of cytokines (including TNF α , IL-6, IL-1 β , and IL-10) have been observed to bypass the translational block; however, the status of Type I IFNs was unclear in these studies [43, 44]. We recently show that infection-driven IFN responses are absent during the hours of pyroptotic cell death. Additionally, exogenous addition of IFN at the onset of infection does not affect rate or magnitude of cell death [4]. Nevertheless, IFNAR-deficient macrophages exhibit a significant delay in pyroptosis. This is due to the requirement of constitutive IFN signaling for expression of Guanylate Binding Proteins (GBPs) (Liu et al. Cell Reports *accepted*), a family of Immune-GTPase necessary for disruption of intracellular bacteria upstream of cytosolic immune sensors [30, 45, 46].

Of note, many viruses, such as Hepatitis C virus and Yaba-like disease virus, have evolved mechanisms to suppress the host IFN response, where every step of the IFN circuit can be targeted [20, 21, 47]. In the face of pathogens that are able to block a *de novo* IFN response, the intrinsic IFN status of the host becomes crucial in determining the infection outcome.

Evidence of Constitutive IFN in Healthy Individuals and Murine Models

How can a cell possess an IFN activation status prior to encountering pathogenic microbes? The answer lies in the presence of **constitutive or tonic IFN signaling** during homeostasis. Amongst the 2000 ISGs that are responsive to IFN receptor signaling, the bioinformatics analysis of 1,398 human and mouse datasets revealed that expression of 200–300 genes in resting B lymphocytes and macrophages that are dependent on IFNAR presence [13], attesting to a role of constitutive IFN at baseline. Notably, the concept that IFNs exist at steady-state first surfaced in the early 1980s. With the IFN protein products being elusively small in quantity and too dilute to be detectable in healthy animals and humans, early studies turned to the hybridization of mRNA transcripts of *Ifn* genes to measure steady-state expression. These studies found varying degree of expression in type I IFN genes in the spleen, kidney, liver, and peripheral blood leukocytes from healthy humans [48, 49]. Indeed, injection of neutralizing antibodies against IFN γ or pan IFN α/β into the peritoneal cavity of mice enhanced tolerance to transplanted tumor grafts [50, 51], indicating that *in vivo* levels of IFNs, although under the limit of protein detection, remains capable of conferring protection against malignant cells.

One of the earliest proposed sources for constitutive IFN production stems from triggering of low-levels of immune activation by commensal microorganisms. For instance, macrophages from C3H/HeJ mice, which are hyporesponsive to LPS due to a mutation in TLR4, were found to be more supportive of vascular stomatitis virus (VSV) replication [52].

In contrast, the restriction to VSV replication by macrophages from the LPS-responsive mouse strain C3H/OuJ can be partially lifted by neutralizing antibodies against IFN α/β . Recently, oral antibiotic treatment of C57BL/6J mice was found to increase susceptibility to LCMV and influenza virus. In this study, the disruption of commensal bacterial population in the gut was associated with severe reduction in expression of *Ifn* genes as well as genes in the IFN signaling pathway including *Stat1/2* and *Irf3/7*, and crucial ISGs involved in antiviral immunity including *Mx1/2* and *Oas* genes [53] (Key Figure, Figure 1). Commensal microbiota is thus one source of constitutive IFN production that may have a protective effect against pathogens [54]. Experiments in pathogen-free mice could help evaluate the contribution of the gut microflora in the induction of constitutive interferon (See Clinician's Corner, box 2).

Self-Ligands Can Drive Constitutive IFN Production During Cellular Homeostasis

In recent years, the discovery of cytosolic DNA sensing pathways has revolutionized our understanding of how intracellular pathogens can elicit an IFN response. Cyclic GMP-AMP synthase (cGAS) is a nucleotidyl transferase that, upon binding to double-stranded DNA, produces a second messenger 2'3'cGAMP, which then activates the adaptor protein STING to elicit TBK1/IRF3 activation and *Ifn* induction [55–57] (Key Figure, Figure 1). Thus, during infection, transfer of pathogen-derived DNA to the cytosol of the host cell, either intentionally or inadvertently, will trigger the activation of cGAS and STING, resulting in *Ifn* induction.

In 2012, homozygous deletion of *Sting* rescued the embryonic lethality of mice that lacked *DnaseII*, a nuclease necessary for the proper digestion of DNA in lysosomes [58]. *DnaseII*^{-/-} *Ifnar*^{-/-} animals are also rescued from embryonic lethality, indicating that in the absence of proper DNase activity, excessive self-DNA activates STING, resulting in IFN-driven lethality [59]. Similarly, mice deficient in TREX, a 3' exonuclease that digests cytosolic DNA, results in autoimmune disease marked by IFN over-production via the cGAS/STING pathway [60, 61]. STING mediated *Ifn* induction was also seen in models of radiation-induced DNA damage and defects in DNA repair (*Atm*^{-/-}) [62]. The same study showed that macrophages experiencing low-levels of DNA damage *ex vivo* were protected against VSV and HSV infections along with enhanced innate PRR responses toward *L. monocytogenes* [62]. Moreover, aberrant exposure of mitochondrial DNA in mice with DNase2-deficiency has been suggested to provide an additional source for cGAS/STING activation and heightened cellular IFN signature -likely leading to the heightened ISGs levels - that is associated with protection in mice against viral infections such as VSV (Vesicular Stomatitis Virus) [27, 63]. Thus, elevated constitutive IFN could be beneficial to the host for some infections.

The cGAS/STING pathway, originally discovered for its IFN-inducing role during infection [64], thus appear to be more commonly engaged during homeostasis to establish and maintain steady-state IFN production. Indeed, our lab and others have observed global reductions in ISG signature in cells deficient for STING or cGAS [5, 27]. Further

investigation of constitutive IFN- signaling in evolutionary divergent mouse sub-species established that several of the wild- derived inbred lines such as MOLF (*Mus.musculus molossinus*), CAST (*Mus musculus castaneus*), and PWK (*Mus musculus musculus*) have very low levels of constitutive IFN as compared to C57BL6 mice. These data suggest that constitutive IFN-signaling was not under selective evolutionary pressure and is dispensable to some extent. In 2009, an *Ifnb*-promoter driven luciferase reporter *in vivo* revealed the thymus as a tissue with high *Ifnb* activity in the absence of any known perturbations to the animal [65]. First, genetic rearrangements in the TCR (T-cell Receptor) locus during thymocyte maturation result in the double strand DNA breaks, which might be one source of tonic IFN in the thymus. In this regard, one would predict that RAG-KO mice should exhibit lower levels of the constitutive interferon in the thymus. Second, the thymus is a site of rapid cellular turnover in which T cells undergo positive and negative selection, high transcriptional activity of *Ifnb* in the thymus supports the hypothesis that cellular damage provides a signal for IFN induction. Do all cells constantly produce cGAS/STING mediated constitutive IFN, or just a few cells in random order exceed a threshold for STING activation and spontaneously secrete paracrine IFN? The latter seems to be more likely alternative because of a gene-dosage effect that has been reported for STING, thus suggesting the existence of a certain threshold of DNA/cGAMP required for STING activation. To distinguish between these scenarios, one could use gene expression analysis of IFN-signaling at the single cell level. Thus, we propose that - in order to maintain constitutive IFN signaling - few random tissue resident cells produce a “spike” of IFN in response to a cytosolic DNA accumulated in the cell above the threshold of sensitivity, which suggests that the mechanism of induced and constitutive IFN is the same in quality but different in quantity of response. Notably, in healthy humans, amniotic fluid was one of the first sites found to harbor detectable amount of IFN α protein [66, 67], further supporting the notion that sites of high cellular or tissue turnover may trigger IFN production *in vivo*. Self-derived signals, in addition to microbiota, can thus drive intrinsic IFN production in healthy animals to establish a low, possibly protective, IFN status.

Tuning Endogenous IFN Status to Ameliorate Autoimmune Disease Manifestation

In contrast to the undetectable concentrations of IFN in healthy humans, high concentrations of circulating IFN α / β is a hallmark of several autoimmune diseases including **Systemic Lupus Erythematosus** (SLE) [68, 69] and several dermatologic disorders including **psoriasis** [70, 71], **Sjögren’s syndrome** [72], **scleroderma** [73] and **dermatomyositis** [74]. One of the key drivers of pathology during autoimmunity is the presence of antibody toward self- antigens (autoantibodies). Notably, many of these autoantibodies target nuclear components, supporting the hypothesis that improperly digested DNA may be triggering nucleic acid sensing pathways to induce high levels of IFN. In the case of interferonopathies including **Aicardi- Goutières syndrome** (RNase or DNase deficiency) and **STING-associated vasculopathy with onset in infancy (SAVI)** (hyperactive STING), excessive interferon directly drives disease [75, 76]. Additionally, reactivation of endogenous **retroelements** can activate the cytosolic RNA sensing pathway RIG-I/MAVS in B cells, resulting in T-cell independent autoantibody production in addition to heightened IFN

signaling [60, 77]. Of note, MAVS has also been shown to be activated by reactive oxygen species from the mitochondria in a virus-independent manner [78], indicating that in addition to their traditional pathogen recognition roles, PRRs may also function to monitor other types of cellular stress.

In a mouse model of SLE resulting from TLR7 overexpression, deletion of IFNAR in these mice reduces disease severity, including serologic and histologic disease manifestations and extended survival, indicating a detrimental role for elevated IFN signaling [79]. Concordantly, JAK/STAT inhibition to block IFNAR signaling in human patients has been shown to be protective during autoimmunity including SLE [80, 81]. On the other end of the spectrum, a subset of **Multiple Sclerosis** patients display low constitutive IFN signature as defined by ISGs mRNA expression in resting peripheral mononuclear cells [82, 83] and serum cytokine levels [84]. In MS patients, recombinant IFN β treatment restores the ISG signature to that of normal patients. The IFN status restoration as supported by increased phosphorylation of STAT1 and elevated expression of ISGs, is accompanied by ameliorated disease in these patients [76]. Aberrance in IFN *in vivo*, either elevated or depressed, thus appears to be a driver of pathology, attesting to the importance and sensitivity of balanced IFN signaling during homeostasis.

Interferon in Cell Death and Tissue Injury

In addition to the above mentioned IFN-associated pathologies, a necrotic form of cell death termed necroptosis was recently shown to require IFN signaling [3, 10, 85]. In cell culture, necroptosis is driven by TNF α [86] or TLR [87] stimulation under conditions of caspase inhibition [88]. For instance, in TLR 3/4 -induced necroptosis, engagement of **TRIF (TIR-domain-containing adapter-inducing IFN)** elicits IFN production while activating RIP1/3 in mouse BMDMs (Bone Marrow Derived Macrophages) [87, 89, 90]. Notably, blocking IFN signaling with anti-IFNAR antibodies at the time of necroptosis induction had no effect on cell death, whereas blocking IFN signaling in the cell culture prior to inducing necroptosis completely abolished cell death, indicating that necroptosis depends on the pre-established IFN status of the cell rather than the reactionary IFN produced in response to the death-inducing trigger. In fact, we found that Mixed Lineage Kinase Like (MLKL), the protein that forms a membrane channel during necroptosis, requires constitutive IFN for maintenance of steady-state protein product [4]. Thus, in addition to previously mentioned caspase-11 driven pyroptosis, cell-intrinsic IFN signaling controls rate of necroptosis by regulating the expression of MLKL (Box 1).

Recent reports defined a role for necroptosis in kidney **ischemic reperfusion injury** [91], in which IFNAR-deficient mice showed mild protection from ischemia reperfusion injury of the kidney [92] and liver [93]. Interferon induction is not observed in the case of kidney injury and the evidence for IFN induction in liver injury is inconclusive. If IFNAR-deficient animals are indeed protected from ischemia reperfusion injury (IRI) in the absence of IFN induction, this suggests that constitutive IFN might be driving tissue injury. IRI presents one of the biggest challenges to successful kidney transplantation, where autoimmunity has been associated with high prevalence for kidney transplant rejection [94, 95]. One theory is that elevated IFN in the transplant recipient sensitizes the kidney to ischemia reperfusion injury.

Furthermore, the endogenous IFN status of certain cancers can be predictive for metastasis free survival and response to chemotherapy, where loss of constitutive IFN signaling in tumors has been associated with resistance to anthracyclines (chemotherapeutics) [96]. Thus, cell-intrinsic IFN status may play a crucial role in the cell death decisions upon encountering external stressors, including infection, hypoxia, and chemical insult.

Concluding Remarks

Infection-elicited IFN spikes frequently mask the underlying effects of constitutive IFN [97]. Despite so, emerging evidence suggests that some of the effects attributed to induced IFN are observed much earlier than IFN induction itself. In these cases, constitutive IFN should be considered for the observed phenotypes. We propose that host-intrinsic IFN signaling determine the abundance of various families of ISGs, pre-determining cellular response upon encountering pathogen or sterile stresses. For instance, constitutive IFN signaling sustains the abundance of key proteins within both the pyroptotic and necroptotic cascades [4, 5]. In cases of infection, the evolutionary advantage of constitutive IFN signaling may be to ensure that some components of IFN-dependent anti-microbial mechanisms can occur without delay and hindrance from the pathogen's efforts at blocking *de novo* IFN synthesis or feedback. Accordingly, the possible role of constitutive IFN in other forms of infection-induced cell death should also be evaluated. There is no doubt that further elucidation of the contribution of constitutive IFN will advance our understanding of its physiological importance and in identification of critical downstream effectors that could be specifically targeted during treatment of various pathologies, including infections, cancers, and autoimmune diseases (See Outstanding Questions).

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Box 1**Tool Box to dissect the contribution of host-intrinsic IFN and stimulus-induced IFN**

1. Models with genetic ablations for components of the IFN signaling pathway may have defects in constitutive IFN status.
 - *Ifnar*^{-/-} and *Ifnb*^{-/-} animals are globally deficient for IFN signaling. The contribution of constitutive IFN can be restored in *Ifnb*^{-/-} cells by addition of exogenous recombinant IFN-I proteins.
 - In macrophage cultures, loss of cGAS or STING results in reduced ISG signature. The extent of the defect is only starting to be characterized.
2. Use of IFNAR blocking antibodies and JAK inhibitors to selectively reduce IFN signature of the cells prior to stimulation or during stimulation.
 - By inhibiting IFNAR signaling for specific periods of time, one can temporally separate pre-established IFN status from IFN induced by the experimental stimulus.
 - Inhibition of IFNAR for 20 or more hours results in the global reduction in ISGs, which can be monitored by STAT1 protein levels and *Isg15*, *Irf7*, *Mx1* mRNA levels.
 - JAK1/2 inhibitors such as Ruxolitinib and Baricitinib are frequently used to block IFNAR signaling in human cells.
3. Quantifying the IFN response by measuring IFNAR signaling efficacy.
 - IFN induction should be quantified on both the mRNA and protein levels, especially since pathogens may be actively hindering the multitude of steps from transcription of *Ifn* to activation of IFNAR.
 - Following STAT1 phosphorylation over time is a sensitive and specific read-out for whether cells are responding to bioactive IFN, independent of gene induction responses that may result from pathways other than IFNAR activity.
 - Monitoring ISGs upregulation and expression indicates progression of the signaling pathway downstream of IFNAR activation to transcription. *Isg15*, *Irf7*, and *Mx1* expression are frequently utilized to monitor the constitutive IFN status of a cell population. One caveat to this approach is that some IFN-responsive genes may have additional promoter elements for binding by other IRF homo- and hetero-dimers that can be activated by signals other than IFNAR-mediated STAT1/STAT2/IRF9 activity.
4. Look for discrepancies between IFN induction and the requirement for IFNAR presence.

- In cases where *de novo* IFN production or signaling is not observed, but the phenotype in question requires IFNAR, consider constitutive IFN signaling as an explanation.

Box 2**Clinician's Corner**

1. High serum interferon concentrations are associated with many common autoimmune diseases including Systemic lupus erythematosus (SLE) and Sjogren's syndrome (SS). Such patients may be sensitized to excessive necrotic cell death in response to ischemic injury. Anti-interferon therapy might be considered for autoimmune patients with ischemic injury.
2. Interferon therapy is used for hepatitis treatment, which will pre-dispose patient cells to necrotic cell death and may explain their sensitization to autoimmunity.
3. Sites of interferon injection are associated with necrotic lesions in certain patients, treatment with necrostatins might prevent cell death. If interferon therapy un-intentionally sensitizes cells to necrotic cell death, then it might be possible to block cell death pathways with necrostatins with interferon treatment.
4. Anti-interferon therapy is in clinical trials for SLE and may have potential for ischemic reperfusion injury to limit cell death via necroptosis.
5. The gut microbiota may be a source of constitutive IFN in human patients. Excessive antibiotic use may ablate the microbiome, reducing the IFN status and pre-disposing patients to pathogenic viral or bacterial infections.
6. Cytokines are not just produced in response to infection or autoimmunity. They are present at low concentrations to keep the host on alert in case of a new infection. Therefore, patients with immunodeficiency might have defects in constitutive cytokine signaling.

Apoptosis is a process of programmed cell death that occurs in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death.

Constitutive IFN – also, tonic IFN, has been first characterized in the early 1980's, is produced constitutively in rested cells in the absence of the pathogen-induced response

Interferons (IFNs) are a group of signaling proteins made and released by host cells in response to the presence of several pathogens, such as viruses, bacteria, parasites, and also tumor cells. They are typically divided among three classes:

Type I IFN: all type I IFNs bind to a specific cell surface receptor complex known as the IFN- α/β receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains; the type I IFNs present in humans are IFN- α , IFN- β , IFN- ϵ , IFN- κ and IFN- ω . They are produced by fibroblasts and monocytes.

Type II IFN: This is also known as IFN- γ or immune interferon and is activated by Interleukin-12. Type II IFNs are released by Cytotoxic T cells and T helper cells, type I specifically. IFN-II binds to IFNGR, which consists of IFNGR1 and IFNGR2 chains and has a different receptor than type I IFN.

Type III IFN: Signal through a receptor complex consisting of IL10R2 (also called CRF2-4) and IFNLR1 (also called CRF2-12). Although discovered more recently than IFNs-I and II, recent information demonstrates the importance of Type III IFNs in some types of virus or fungal infections.

IFNAR signaling pathway: Interaction of IFN with their specific receptors promotes association of activated receptors with STATs (Signal Transducer and Activators of Transcription) (STATs) and JAK (Janus kinase) kinases followed by phosphorylation of STAT1 and STAT2 by Jak. As a result, an IFN-stimulated gene factor 3 (ISGF3) complex forms—this contains STAT1, STAT2 and a third transcription factor called IRF9—and moves into the cell nucleus. Inside the nucleus, the ISGF3 complex binds to specific nucleotide sequences called IFN-stimulated response elements (ISREs) in the promoters of certain genes, known as IFN stimulated genes ISGs.

Legionnaires' disease is a form of atypical pneumonia caused by any type of Legionella bacteria. Signs and symptoms include cough, shortness of breath, high fever, muscle pains, and headaches

Nucleic Acid Sensors – a family of sensors – such as RIG-I (Retinoic Acid Induced Gene) and MDA5 (Melanoma Differentiated Gene 5) - recognizing cytosolic ds RNA. Cytosolic DNA is recognized by cGAS (Cyclic GMP-AMP synthase), IFI-16 (Gamma Interferon Inducible Protein 16), DDX41 (Dead Box Helicase 41), and AIM2 (Absent in Melanoma 2)**Necroptosis** is a programmed form of necrosis, or inflammatory cell death. Conventionally, necrosis is associated with unprogrammed cell death resulting from cellular damage or infiltration by pathogens, in contrast to orderly, programmed cell death via apoptosis. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global[vague] mRNA decay.

Pattern recognition receptors (PRRs) - are germline-encoded host sensors, which detect molecules typical for the pathogens. PRRs play a crucial role in the proper function of the innate immune system. PRRs

Pyroptosis is a highly inflammatory form of programmed cell death that occurs most frequently upon infection with intracellular pathogens and is likely to form part of the antimicrobial response. Unlike apoptosis, cell death by pyroptosis results in plasma-membrane rupture and the release of damage-associated molecular pattern (DAMP) molecules such as ATP, DNA and ASC oligomers (specks) into the extracellular milieu, including cytokines that recruit more immune cells and further perpetuate the inflammatory cascade in the tissue.

Scleroderma is an autoimmune disease that affects skin, blood vessels, and internal organs. The disease is caused by genetic and environmental factors with mutations in HLA genes playing a crucial role in the pathogenesis. It begins with an inciting event at the level of the vasculature, probably the endothelium. The inciting event caused by viral infection or oxidative stress results in endothelial cell damage and apoptosis that manifests in early clinical stages as tissue oedema. Scleroderma is predominantly a Th1 and Th17-mediated disease.

Systemic lupus erythematosus (SLE), also known simply as lupus, is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue in many parts of the body. Symptoms vary between people and may be mild to severe. Research indicates SLE may have a genetic link. SLE does run in families, but no single causal gene has been identified.

Sjögren syndrome is a long-term autoimmune disease in which the moisture-producing glands of the body are affected. This results primarily in the development of a dry mouth and dry eyes. Other symptoms can include dry skin, vaginal dryness, a chronic cough, numbness in the arms and legs, feeling tired, muscle and joint pains, and thyroid problems.

Scleroderma is a group of autoimmune diseases that may result in changes to the skin, blood vessels, muscles, and internal organs. The disease can be either localized to the skin or involve other organs in addition to the skin.

STING – Stimulator of Interferon Genes (encoded by *TMEM173*), an adaptor mediating responses to cytosolic DNA, a crucial regulator of innate immune responses triggered by pathogen-derived cytosolic DNA. In addition, STING responds to self-DNA that enters into the cytosol as a result of mitochondrial and genotoxic stresses. Activation of STING leads to production of type I IFN. STING directly recognizes second messenger di-nucleotides such as ci-di-AMP.

STING-associated vasculopathy with onset in infancy (SAVI) - neonatal onset systemic inflammation with severe cutaneous vasculopathy leading to extensive tissue loss caused by de novo mutations in *TMEM173* encoding STING.

TLRs – Toll-Like Receptors, a family of PRRs that are expressed on the membranes of leukocytes including dendritic cells, macrophages, natural killer cells, cells of the adaptive immunity (T and B lymphocytes) and non immune cells (epithelial and endothelial cells, and fibroblasts). The TLRs recognize molecules that are broadly shared by pathogens.

Outstanding Questions

- Two potential *in vivo* sources of constitutive IFN include the thymus and the developing embryo, suggesting that cellular turnover may be a source of IFN induction during homeostasis. Ablation of commensal microbiota was also shown to reduce pathogen induced IFN response, suggesting non-sterile tissue sites to be another source for IFN production during normal physiology. **What are other sources of constitutive IFN *in vivo*, during development and adulthood?**
- The average peak of ISG induction centers around 2 hours post IFNAR stimulation and the rate of translation of ISG mRNA to functional protein is unknown. **For specific pathogens, what is the timing of the pathogen-driven IFN response to confer protection to the host at the cellular and tissue levels?**
- IFN-activated status of cells sensitizes them to death in response to infection, hypoxia, and chemical insults. **How do increased or reduced levels of host-intrinsic IFN status impact responses to infection and sterile stressors in various autoimmune pathologies?**
- As we gain increased knowledge on the individual ISGs and ISG networks that drive anti- microbial and cell death responses, **can our understanding of host-intrinsic ISG networks be used to build predictive models to treat infections and modulate tissue stress and injury?**

Highlights Box

- Low activation levels of cGAS/STING during DNA damage increases baseline IFN signaling and enhances cellular response to viral and bacterial infection, indicating a protective role for constitutive IFN.
- Mitochondrial damage releases DNA into the cytosol, the ensuring activation of cGAS/STING has been shown to induce an IFN response that result in pathology or lethality, thus implicating elevated host-IFN status with cell death and tissue injury.
- The responsiveness of tumors to chemotherapy appears to depend on the tissue's own IFN signature, suggesting that cellular IFN status controls how an external stressor is perceived and responded to by the cell.
- IFN status is associated with multiple autoimmune phenotypes: elevated IFN contributes to pathogenic mechanisms in SLE, where IFNAR blocking has been shown to alleviate disease symptoms. In contrast, a subset of MS patients present low constitutive IFN, and recombinant IFN treatment ameliorated their disease manifestation.

Figure 1, Key Figure. Sources of Constitutive IFN in maintaining the “Constitutive Isg-ome”
In vivo, multiple triggers exist to stimulate low levels of IFN signaling, thereby generating a cell- intrinsic IFN activation status during homeostasis. The microbiota of the organism acts as an endogenous IFN trigger, presumably through activation of TLRs 3 and 4 -both signal through TRIF to activate IRF3 for IFN induction. Intracellular Pattern Recognition pathways can also receive signals from self-derived DNA, RNA, and stress signals. Undigested DNA from lysosomes, mitochondrial DNA (mtDNA) exposed during mitochondrial turnover, and damaged DNA from the nucleus can all activate the cytosolic DNA sensing pathway cGAS/STING to induce IFN via IRF3 activity. Under conditions of cellular stress, reactivation of endogenous retroelements can generate RNA species to activate the cytosolic RNA sensing pathway RIG- I/MAVS. Reactive oxygen species (ROS) from stressed mitochondria can furthermore trigger MAVS signaling in the absence of viral ligands. MAVS activation results in IRF3 transcription of *Ifn* genes. IRF3 is thus a central player in the endogenous production of IFN during homeostasis.

Post IFN production, the cytokine binds to dimeric IFNAR receptors on the cell surface in an autocrine or paracrine manner, activating transcription factors STAT1/STAT2/IRF9 to initiate transcription of ISGs. Constitutive IFN signaling thus generates a baseline IFN signature in the cell, referred to in the diagram as “Constitutive Isg-ome”, amongst which are components within the IFNAR signaling pathway including STAT1, STAT2, and IRF9. Understanding which genes/proteins are present within the “Constitutive Isg-ome” will significantly aid our understanding of the mechanism behind rapid cellular responses to stress and infection.