Published in final edited form as:

Trends Immunol. 2021 February ; 42(2): 151–164. doi:10.1016/j.it.2020.11.006.

Early Events Triggering the Initiation of a Type 2 Immune Response

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Abstract

Type 2 immune responses are typically associated with protection against helminth infections and also with harmful inflammation in response to allergens. Recent advances have revealed that type 2 immunity also contributes to sterile inflammation, cancer, and microbial infections. However, the early events that initiate type 2 immune responses remain poorly defined. New insights reveal major contributions from danger-associated molecular patterns (DAMPs) in the initiation of type 2 immune responses. In this review, we examine the molecules released by the host and pathogens and the role they play in mediating the initiation of mammalian innate type 2 immune responses under a variety of conditions.

Hallmarks of Type 2 Immune Responses

The mammalian type 2 immune response is dependent on extrinsic and intrinsic signals sensed by the host. However, the stimuli and associated mechanisms influencing the course of the response remain unclear, particularly regarding the initiation of the innate type 2 immune response. This response typically includes upregulation of interleukin-4 (IL-4), IL-5, IL-9, and IL-13, collectively referred to as type 2 cytokines [1,2]. Cells mediating mammalian type 2 innate immunity include macrophages, group 2 innate lymphoid cells (ILC2s), neutrophils, mast cells, eosinophils, and basophils. The innate immune response provides an immune environment required for subsequent activation of adaptive immunity. In the type 2 immune response, adaptive immunity is mediated by $CD4^+$ T helper (Th)2 cells producing large quantities of type 2 cytokines and B cells which secrete IgE and IgG antibodies, and also various cytokines [1,2]. Both innate and adaptive immune cells express shared and lineage-specific activation signatures, largely dependent on IL-4 receptor signaling [3]. The specific tissue in which the response occurs, including associated non-immune cells, such as epithelial cells, and the associated tissue matrix can also contribute substantially to this response [3]. Parasitic worms, or helminths, comprise the major group of pathogens consistently triggering polarized type 2 immune responses, but at least

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Disclaimer Statement

G.H. owns stock in Purine Pharmaceuticals, Inc. and has patents related to purinergic signaling in sepsis.

elements of this type 2 immune response are elicited by other pathogens, allergens, and also by inert sterile insults, including microparticles and trauma [3]. Of note, type 2 immune responses can also be induced during microbial infections where **type 1 immune responses** (see Glossary) usually predominate and can influence the host's immune response as being either protective or associated with harmful inflammation [3]. Various pathogens can also induce **type 17 immune responses**, which can be associated with either type 1 or 2 immune responses [3].

The innate immune response can recognize foreign invaders such as viruses, bacteria, fungi, and parasites, in part by binding **pathogen-associated molecular patterns (PAMPs)** through **pattern recognition receptors (PRRs)**. PRRs also recognize specific molecules released from stressed, damaged, or dying cells, referred to as **danger-associated molecular patterns (DAMPs)** [4]. These DAMPs may also have other important activities in cellular function, but when released in the context of an infection or even sterile insult, may trigger innate immunity (Box 1). Three epithelial cell-derived cytokines, thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, were recently identified as important in 'alarming' type 2 immune responses. These **cytokine alarmins** are released by epithelial cells and a variety of other non-immune and immune cell types [5]. In some cases, they can play distinct and essential roles in driving type 2 immunity, while in other cases they appear to have more redundant roles [6]. There are excellent reviews discussing how these molecules might stimulate type 2 immune responses [5,7–9]. This review focuses on recently identified factors and associated signaling pathways upstream of these three cytokine alarmins, triggering innate type 2 immunity during helminth infection, sterile immunity, allergens, and cancer. This analysis reveals the ubiquitous nature of type 2 immunity and the diverse stimuli inducing its development in different tissue microenvironments.

Helminth Infections

Large multicellular helminths can trigger potent and polarized type 2 immune responses. Considerable advances in our understanding of the initiation phase of the immune response to helminth infections have been made over the past few years, using experimental models of helminth infection, including *Heligmosomoides polygyrus bakeri (Hpb), Schistosoma mansoni (Sm), Nippostrongylus brasiliensis (Nb), Trichinella spiralis* (*Ts*), and *Trichuris muris (Tm)* [10]. DAMPs are often released by damaged host cells, as a consequence of these large multicellular parasites trafficking through tissues and causing cellular damage by their sheer physical size and release of proteases. Several DAMPs shown to trigger innate immunity in the context of helminth infection include ATP, chitinase-like proteins, and trefoil factors. In many cases each of these different stimuli are essential for the development of this type 2 immune response, indicating the high threshold required for its initiation.

ATP

ATP functions as a biological energy currency, but when released by stressed or dying cells ATP can also promote initiation of type 2 immune responses. Recent studies have shown that after *Hpb* infection in mice, extracellular ATP (eATP) is released by apoptotic intestinal epithelial cells (IECs) [11]. eATP can bind the type 2 purinergic receptor, $P2X_7$ on mast

cells, leading to their activation, IL-33 production, and the development of type 2 responses [11]. ATP can also be catabolized to adenosine by the cell surface ectonucleotidases CD39 and CD73 [12,13], which are elevated in intestinal epithelial lymphocytes following *Hpb* infection in mice [14]. Furthermore, A_{2B} adenosine receptor (A_{2B}AR) deficient mice ($A_{2B}AR^{-/-}$) have impaired type 2 immunity and delayed worm expulsion relative to wild type (WT) mice [14] (Figure 1). Whether adenosine is derived from eATP or other sources remains unclear. Taken together, these studies indicate that ATP and its metabolites can function as essential DAMPs in driving helminth-induced immune responses. However, the mechanism of ATP release, the potential role of other cell sources of ATP, and whether extracellular and/or intracellular ATP/adenosine interactions initiate type 2 immune responses remains unclear.

Trefoil factors

Trefoil factor family (TFF) members 1–3 are epithelial protease-resistant proteins expressed in humans and mice that have protective functions and are involved in wound healing but also can contribute to the initiation of the type 2 response [15,16]. TFF2, a molecule secreted by epithelial cells, stimulates the release of IL-33 from lung and gut epithelial cells to promote type 2 immunity during *Nb* infection in mice [16]. TFF3 secreted by goblet cells enhances epidermal growth factor receptor (EGFR) signaling through the disruption of leucine-rich repeats and Ig-like domain-containing nogo receptor-interacting protein 2–EGFR complexes. This disruption leads to EGFR signaling and enhancement of type 2 immune responses to *Hpb* and *Nb* infections in mice [17,18].

Chitinase and Chitin

Chitins are polysaccharides that help form the protective barriers of many fungi and the exoskeletons of insects and parasites. Chitinases are chitin-degrading enzymes, expressed by epithelial cells and macrophages. They are likely important in digestion, and recent studies suggest they may prime type 2 responses to helminths [19,20]. Epithelial-cell-derived acidic mammalian chitinase (AMCase) can initiate type 2 immune responses in the gastrointestinal tract of mice during *Nb* and *Hpb* infections [20]. Epithelial-cell-derived cytokines, TSLP, IL-25, and IL-33, released in response to chitin stimulation, can induce ILC2s to produce IL-5/IL-13, potentially driving eosinophilia and accumulation of **alternatively activated (M2) macrophages** in murine lungs [21]. By contrast, chitinase-like proteins (CLPs) share homology with chitinase but are enzymatically inactive. During infection of mice with *Nb*, the CLP Ym-1 is released as larvae invade the lungs, inducing the expansion of γδ T cells and their production of IL-17 [22]. IL-17 then recruits neutrophils to the lungs, contributing to acute lung injury in the infected mouse [23]. IL-17 can also suppress early interferon (IFN)γ production in mice, and potentially have other effects which promote type 2 responses [24–26]. Thus, although DAMPs play a major role in driving type 2 immunity, chitin, and potentially other helminth-derived molecules may function as PAMPs, contributing to the overall response.

Early Cytokines

Type 1 IFNs (IFN-Is) are cytokines secreted by infected cells and through their binding to their specific receptor, induce the transcription of IFN-stimulated genes (ISGs), leading to a protective response during viral and certain bacterial infections [27]. Recent work has shown that IFN-Is may also play an important role during initiation of type 2 immunity. Specifically, using *Sm* egg antigen injection as a model to induce type 2 immune responses in mice, IFN-I signaling was required to drive dendritic cell (DC) activation, migration, and localization with $CD4^+$ T cells, in the draining lymph nodes in WT mice compared with *Ifnar1*$^{-/-}$ mice [28]. In another study, IFN signaling promoted Th2 cell activation after *Nb* infection: IFN-I signaling in DCs from *Nb*-infected mice promoted the optimal activation of IL-4-producing $CD4^+$ T cells. However, blockade of IFN-I signaling, by administering anti-interferon (α and β) receptor (IFNAR) antibody at the time of *Nb* inoculation, did not affect DC migration, nor DC antigen transport into the skin draining lymph node [29]. Thus, though not yet well-defined, type I IFNs and IL-17 need to be included as potential early players in the initiation of type 2 responses.

Parasite Products

Excretory/secretory (ES) products produced by helminths are diverse and can modulate various components of the host immune response. Recent studies suggest that ES products induce production of the neuropeptide neuromedin U (NMU) by neuronal cells; NMU then binds the receptor NMUR1 on ILC-2s in mice, driving IL-5 and IL-13 production [30,31]. In the intestine, ES products likely activate the **tuft cell** to promote type 2 responses (Box 2). Furthermore, the helminth secretory product, IL-4-inducing principle of *Sm* eggs (IPSE)/α-1, can induce the production of IL-4 from basophils via IgE receptor crosslinking in mice [32]. In contrast, Omega-1, a ribonuclease also produced by *Sm*, suppresses Th1 responses and conditions mouse DCs to polarize $CD4^+$ T cells into Th2 cells and regulatory T cells [33–35]. ES molecules also add a significant regulatory component to the helminth-induced type 2 immune response, which may modulate initiation of the response [36].

Early Innate Cell Interactions

Various innate immune cells contribute to the initiation of type 2 immune responses to helminth infection. Neutrophils are the most abundant leukocytes in circulation and during *Nb* infection in mice, neutrophils swarm L3 larvae immediately after they penetrate the skin, deploying **neutrophil extracellular traps (NETs)** capable of damaging the larvae [37]. During *Nb* infection, neutrophils can also promote M2 macrophage differentiation, with recent studies suggesting the involvement of macrophage **efferocytosis** of apoptotic neutrophils [38] and also neutrophil production of cytokines, including IL-13 [39]. In further studies, **surfactant proteins** also promoted M2 macrophages during helminth infection, suggesting an important role for the cellular matrix in the tissue microenvironment in shaping macrophage differentiation and the associated type 2 response [40,41]. Basophils and eosinophils are recruited to the lung tissue after primary infection with *Nb* and can produce IL-4 and IL-5 [42,43]. Basophils can also play a role in the initiation of type 2 immune responses during secondary infection to *Nb* in mice, where they can promote the alternative activation of macrophages through the production of IL-4 [44]. In addition, a

mouse dermal DC subset expressing CD301b primes $CD4^+$ T cells in the draining lymph nodes to produce IL-4, favoring the initiation of type 2 immune responses in *Nb* infections in mice [45]. Also, in humans and mice, ILC2s can produce IL-5 and IL-13, and are thus potentially important as early initiators of type 2 immune responses [46–48]. Overall, ILC2s may play an important role in barrier immunity, being activated by skin, lung, and gut epithelial cells after helminth infection in humans and mice [49]. In the context of helminth infection in mice, stromal cell IL-33 release can trigger ILC2 production of IL-5 and IL-13 in white adipose tissue, thereby likely promoting metabolic homeostasis. Specifically, IL-33 was shown to be essential in this process, as cytokine production was blocked when ILC2s lacked the ST2/IL-33 receptor in mice ($Il1rl1^{-/-}$) [50]. However, the actual role of ILC2s in driving type 2 immunity in humans and in intact mice remains unclear, raising the possibility that they might have other effects, which remain to be assessed [51]. Specific interactions between these different innate immune cell populations and also their communication with non-immune cells and effects of signals produced by the tissue-specific cell matrix remains understudied and are likely to become a major focus of future research.

Sterile Inflammation

Sterile inflammation (SI) occurs in response to mechanical trauma, irritants, cell death, tissue injury, or chemically induced injury in the absence of pathogenic microbes. Numerous particulates have been identified that promote SI including: uric acid (UA), silica crystals, aluminum salts, asbestos, pollutants, prosthetic implant wear debris, and cholesterol crystals [52–56]. SI plays an important role in tissue repair, but dysregulation of this immune response can also lead to chronic inflammatory diseases, such as gout [57]. Although the triggers that initiate SI are poorly understood, a number of recent studies indicate a significant role for type 2 immune responses (Figure 2).

Particle-Induced Inflammation

A diverse group of particulates triggers SI and skews the response towards type 2 immunity. For example, UA crystals, the end product of purine metabolism, which contributes to gout and acute gout arthritis [58,59], is released from dying or stressed cells. UA crystals, produced by airway epithelial cells after internasal house dust mite (HDM) allergen inoculation with ovalbumin (OVA), can directly induce type 2 immunity in mice, as measured by increased production of type 2 cytokines and serum IgE relative to controls. In this model, inflammatory DCs were activated through the spleen tyrosine kinase (Syk) and PI3-kinase δ signaling pathway independently of the NLRP3 inflammasomes [60]. Silica crystals and alum administered to mice could also trigger type 2 immune responses through Syk-dependent and inflammasome-independent pathways leading to the production of prostaglandin (PG)E2 by macrophages and elevated serum IgE [54,60]. Of note, in these studies, microparticles did initially trigger activation of the NLPR3 inflammasome and production of biologically active IL-1β; however, this parallel response was not required for the subsequent development of the type 2 response [54,55,60–62]. Also, particulates, including pollutants such as diesel exhaust particles (DEPs), could trigger potent type 2 responses in mice, enhancing emphysema and allergen-induced inflammation relative to controls [63–65]. Recently, micron-sized particles released as wear debris by prosthetic

joint replacements have been linked to increases in SI and type 2 immunity, resulting in osteolysis and implant failure in humans [62,66]. In peritoneal and knee joint mouse models, sterile metallic microparticles, similar in size and composition to wear debris, stimulate the recruitment of innate immune cells, including neutrophils, eosinophils, and M2 macrophages, resulting in elevated type 2 cytokines relative to controls. These macrophages produce IL-33 through a Syk- and Bruton's tyrosine kinase (BTK)-dependent pathway, which promotes inflammasome-independent initiation of type 2 immunity and requires activation of cell death pathways [62,67].

Sterile particulates, including pollutants, can also enhance inflammation during infectious disease. Recently, silica exposure in the mouse lung induced type 2 immune responses, exacerbating infection by *Mycobacterium tuberculosis* [68]. Associated tissue damage triggered the release of extracellular DNA, which stimulated the cyclic GMP–AMP synthase (**cGAS)/STING (**stimulator of interferon genes) **pathway**, as demonstrated by the increased expression of the STING-coding gene *Tmem173* and the cGAS-coding gene *Mb21d1.* Associated increases in IFN-I promoted harmful type 2 inflammation, which was neutralized in *Ifnar*$^{-/-}$ mice [68]. Taken together, these studies indicated that sterile inert micrometer sized particles of varying composition can trigger type 2 innate inflammatory responses in different tissues. Increased particle exposure in the external environment, ranging from diesel exhaust particles to silica exposure, and also the release of microparticles from various internal prosthetic implants, can potentially impact immune function, and thus, this understudied area merits further attention.

Trauma-Induced Inflammation

Mechanical/hypoxic traumatic injuries can also trigger SI and type 2 immunity. During sterile liver injury in experimental mouse models, necrotic cell release of ATP induced the recruitment of GATA-6^{+} macrophages from the peritoneal cavity. At the site of injury, these macrophages differentiated into M2 macrophages, where they contributed to tissue repair by ingesting necrotic nuclei of cells at the site of injury [69]. In a different study, pericardial fluid samples, taken from patients undergoing valve replacement surgery, were elevated in M2 macrophages, suggesting that they might contribute to cardiac repair after injury [70]. Also, clinical studies of patients with severe blunt force trauma injuries, excluding brain and head traumas, revealed elevated concentrations of IL-33 and type 2 cytokines in human blood relative to controls [71]. As such, type 2 immunity might be a major component of SI resulting from tissue injury caused by a variety of stimuli ranging from solid particulates to mechanical injury.

Allergens

The type 2 immune response plays a major role in allergic inflammation including immediate hypersensitivity reactions. Allergic reactions can be associated with infectious disease or can occur following exposure to allergens under sterile conditions. Allergens include pollen, fungi, animal dander, pollutants, and HDM, many of which can have enzymatic activities contributing to tissue damage. Unlike inert particulates, allergens may

also have epitopes that can activate adaptive immunity, including both T and B cells, exacerbating the inflammatory response to challenges after sensitization [72].

Purinergic Signaling

Recent studies have begun to unravel how allergens stimulate innate type 2 immune responses. Infection with the fungal allergen, *Alternaria alternata,* can drive allergic lung inflammation: airway epithelial cell exposure to this allergen in mice increased calcium signaling and their release of eATP [73]. Subsequent signaling through the cell surface purinergic receptor, $P2Y_2$, triggered the release of full-length IL-33 from airway epithelial cells, inducing the production of IL-5 and IL-13 and a downstream type 2 response in murine lungs [73,74]. *Staphylococcus aureus* serine-protease-like proteins also increase the release of full-length IL-33, through pathways that are independent of either apoptosis or necrosis [75]. Similarly, in OVA peptide and HDM asthma mouse models, eATP signaling through the $P2X_7$ receptor on macrophages stimulates an M2 macrophage phenotype [76]. Adenosine can also contribute to chronic allergic inflammation in mice, as the response to OVA plus alum is attenuated by blocking A_{2B}AR signaling relative to controls [77]. These studies have demonstrated the importance of eATP release and downstream adenosine in driving allergic responses in several different mouse models. Of note, cellular stress triggering calcium flux and eATP release from intact cells might in some cases be sufficient to drive the response in the absence of significant cell death.

Other Signaling Pathways Leading to Allergy

Several other mechanisms besides eATP have been implicated in allergic inflammation. As already discussed, UA crystals can also promote type 2 responses. When released in the airways they can trigger allergic inflammation in HDM-allergen-challenged mice, and increases in UA are correlated with increased allergic inflammation in asthmatic subjects following allergen provocation [60]. Also, pollen proteases damage the epithelial tight junction occludin, claudin-1, and ZO-1 proteins, likely triggering the release of DAMPs and allowing translocation of allergens in the airway epithelium, inducing allergic reactions [78]. In an HDM mouse model, the allergen protease Derp1 of HDM can enhance initiation of the type 2 immune response through cleavage of full-length IL-33 to more active forms, thereby directly promoting type 2 immunity, including activation of ILC2s to produce IL-5 and IL-13 [79]. Of note, full-length IL-33 may be also cleaved extracellularly by enzymes released by both innate immune cells and epithelial cells, resulting in its inactivation, or the formation of more active fragments, which can enhance the initiation of type 2 immunity [80–82]. Additionally, proteases such as papain activate type 2 immune responses by damaging IECs, triggering the release of IL-33 [83]. In another mouse model, alkaline protease (Alp)1 from the fungus *Aspergillus fumigatus* induces damage to epithelial cell junctions of bronchiolar club cells in the lungs. Specifically, this mechanical damage is recognized by transient receptor potential channel 4 on club cells, initiating calcium flux and inducing calcineurin; this promotes IL-5 production by $GATA3^+$ Th cells and eosinophilia, thereby triggering allergic inflammation [84].

Mechanisms other than cell damage may also contribute to the type 2 immune response. In a HDM mouse model of allergy, ILC2 production of IL-13 is instead driven by

NMU, which amplifies IL-25-mediated inflammation by binding its receptor NMUR1 on ILC2s [85] (Figure 1). How neurons and sensory cells sense the allergen and induce the expression of NMU remains unknown. Also, the carbohydrate-binding lectin, intelectin, expressed in humans and mice, is upregulated in airway and skin epithelial cells in OVA or HDM mouse models, and is required for the upregulation of IL-25, IL-33, and TSLP [86–88]. Taken together, several different pathways have now been implicated in triggering allergic reactions. Accordingly, various early signals ranging from eATP to NMU may play essential roles in initiating the type 2 immune responses. Taken together, studying how these different pathways can interact to drive allergic inflammation is an important next step to understand how these allergic responses are initiated. Although they remain understudied, death signaling pathways might also play a significant role in allergic inflammation and thus, this connection might potentially be an important area of future investigation.

Cancer

A hallmark of cancer development is tumor-promoting inflammation, which may occur at various stages of tumorigenesis [89]. Type 1 immunity plays a predominant role during the tumor initiation phase with natural killer (NK) and $CD8^+$ T cells recognizing cancer cells and eliminating them [89]. As the tumor progresses, a type 2 immune response associated with immunosuppression and immune tolerance can develop, resulting in enhanced tumor growth and/or progression, thereby promoting tumor malignancy (Figure 3) [90]. Myeloid cell plasticity is characteristic of this transition [91], as the tumor microenvironment (TME) progressively develops into a complex network of host cells including fibroblasts, endothelial cells, immune cells, and the extracellular matrix, interacting together to support tumor growth [91]. Moreover, tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), monocytic myeloid-derived suppressor cells (M-MDSCs), and DCs are all key innate immune cell players [89].

Tumor-Secreted Factors – Tumorigenic M2 Macrophages

Type 2 immunity is in part initiated within the TME by tumor cell production of colony-stimulating factor (CSF)-1, which promotes M2 macrophage differentiation [92,93]. In a mouse mammary cancer model, CSF-1, expressed by the mammary epithelium, binds CSF1 receptor on macrophages, promoting tumor metastasis in part by regulating macrophage function and infiltration into the TME [94]. In a PyMT mammary cancer mouse model, type 2 immunity is also driven by tumor and stromal cell secretion of CCL2, which induces CCR2-dependent recruitment of M2 macrophages [95]. Activated M2 macrophages, within the TME, secrete vascular endothelial growth factor A (VEGFA), which enhances tumor cell extravasation promoting metastasis [95] and tumor angiogenesis [96,97]. By contrast, tumor necrosis factor (TNF) has antagonized M2 macrophage polarization, as *Tnfrsf1a*$^{-/-}$ mice implanted with thymoma (EG7) lymphomas, show enhanced M2 macrophage polarization, which is further promoted by eosinophils producing IL-13, relative to controls [98]. Thus, a balance between type 1 inflammation associated with TNF, and type 2 inflammation associated with IL-13 and CSF-1 could influence the development of M2 macrophages and associated tumor progression [98]. Lastly, although controversial, M-MDSCs can give rise to TAMs and have been associated with shaping the metabolic state of the TME. This

metabolic axis has been deemed to include lipid, glucose, and amino acid metabolism, which can in turn inhibit antitumor responses [99].

Tumor-Secreted Factors – Tumorigenic N2 Neutrophils

Similar to M2 macrophages, neutrophils can also exhibit a protumor, alternatively activated phenotype [100]. Such neutrophils can express tumor-associated factors that may promote metastasis, carcinogenesis, immunosuppression, and angiogenesis [101–103]. Factors secreted by tumor cells such as tumor-derived CSF-2 within the TME of human gastric cancer patients can activate these neutrophil types [104]. *In vitro* studies have shown that surface expression of programmed death ligand 1 on neutrophils can suppress T cell function *in vitro* [104] (Figure 3). Within the local TME, another study in 4T1 tumor-bearing mice has shown that c-kit$^+$ tumor-elicited neutrophils can prime a low glucose metabolic state that sustains an immunosuppressive type 2 microenvironment while inhibiting a type 1 immune response [105]. This N2 neutrophil phenotype is similar to the N2 neutrophil phenotype that was recently described in the context of helminth infections [39]. It will be interesting in further studies to compare these alternatively activated neutrophils that seem to arise in response to different stimuli and associated tissue microenvironments.

ILC2s: Pro- or Antitumorigenic?

In patients with acute promyelocytic leukemia (APL), type 2 immunity promotes tumor growth and progression, potentially through the involvement of tumorigenic ILC2s [106]. Recent studies using human APL and bladder cancer samples, as well as APL mouse models, suggest that the type 2 immune response may be initiated by the binding of tumor derived factors, such as PGD2 and B7H6, which bind to their receptors CRTH2 and NKp30m, respectively, on ILC2s, driving an IL-13 response and the recruitment and activation of M-MDSCs into the tumor [106,107]. Furthermore, in a mouse B16-tumor-bearing model, ILC2s are rendered protumorigenic by suppressing NK cell IFN-γ production [108]. Conversely, ILC2s have also been reported to be antitumorigenic in specific TMEs. For example, in a B16 melanoma mouse tumor model of decreased lactate dehydrogenase A expression (using small hairpin RNAs complementary to *Ldha* in melanoma cells), stimulation of ILC2s with IL-33 leads to recruitment of eosinophils to the tumor site, thus minimizing tumor growth relative to controls [109]. Thus, recent studies have suggested that the particular TME may influence whether ILC2s are pro- or antitumorigenic.

Other Immune Cells

In a mouse model of pancreatic ductal adenocarcinoma TME, M2 macrophages release CCL7, which promotes the recruitment of basophils secreting IL-4, thus stimulating M2 macrophage recruitment and type 2 inflammatory responses in the tumor-draining lymph nodes [110]. Moreover, in a transgenic mouse model of mammary adenocarcinoma, CD4$^+$ T cell-derived IL-4 stimulates the polarization of TAMs and enhanced EGF/EGFR signaling, resulting in tumor invasion and metastasis, relative to controls [111]. Taken together, these studies suggest that tumor-secreted factors can act as prerequisites for the initiation of type

2 immune responses [91], although their individual roles in initiating type 2 immunity in the TME remain elusive.

Concluding Remarks

Our understanding of the complex network of initiating factors involved in the burgeoning field of type 2 immunity has advanced considerably but remains at an early stage of investigation (see Outstanding Questions). The diversity of stimuli that trigger type 2 immune responses is considerable. However, it is also clear that many common pathways are shared in the initiation of the ensuing response. Overall, DAMPs released because of cell stress or death appear to be generally significant in driving the initiation of type 2 immune responses. In worm infections, even though helminth-secreted ES factors certainly contribute to activation of the immune response, cell damage resulting from these multicellular parasites trafficking through tissues is likely a major factor in the initiation of the response. However, the plasticity of type 2 immune response, including its effects in the context of microbial infections, underscores its previously underappreciated ubiquitous nature. In certain cases, the apparent variation in response to these stimuli might be partly due to what has been initially investigated in different model systems. In this regard, it will be important to conduct more direct comparisons between type 2 responses elicited by allergens, helminths, cancer, particulates, trauma, or other stimuli. Also, the tissue microenvironment might play a significant role in these observed differences. The initiation of the type 2 immune response at barrier surfaces might preferentially depend on specialized epithelial cells and associated innate myeloid and lymphoid cells triggering the response, while non-epithelial responses might utilize different pathways. As an example, a recent study has shown that the initiation of joint and peritoneal type 2 responses to microparticles are dependent on BTK signaling in macrophages, while enteric responses to helminths are BTK independent, and such findings might exemplify such differences [62]. Finally, it also clear that the ubiquitous type 2 response can have beneficial effects associated with tissue repair, versus harmful effects associated with fibrosis and increased susceptibility to infection. What tips the balance in these outcomes may be context dependent, including the tissue microenvironment, the genetic background, as well as previous exposure to pathogenic and commensal microbes and parasites, resulting in a **trained innate immune response** with a bias toward a specific type of response, although this remains conjectural. Further elucidation of the triggers that initiate type 2 immunity could reveal novel checkpoints that might be targeted for future therapies.

Acknowledgments

National Institutes of Health (NIH) grants R01GM066189 (G.H.) and R01DK113790 (W.C.G. and G.H.), R01DK113790 (W.C.G), R01AI131634 (W.C.G), and T32AI125185 to D.W.E.

Glossary

Alternative activated (M2) macrophages:
historically, this term was used to describe macrophages activated by IL-4 and/or IL-10 *in vitro*, with further attempts to categorize according to specific stimulating cytokines. However, macrophage phenotypes are more heterogeneous *in vivo*, as additional stimuli

besides cytokines contribute to their activation. As such, the term M2 macrophage, although still debated, is now often used to describe a generalized macrophage activation phenotype expressing a broad set of shared markers in the context of type 2 immune responses

cGAS/Sting pathway:
self and non-self DNA-sensing nucleotidyl transferase enzyme cyclic GMP–AMP synthase (cGAS), stimulator of interferon genes (STING). Pathway involved in initiating IFN induced responses to the accumulation of cytosolic DNA

Cysteinyl leukotrienes (cysLTs):
lipid mediators synthesized from arachidonic acid and released upon demand by tuft cells, mast cells, eosinophils, basophils, and macrophages. cysLTs initiate immune responses and contribute to maintain tissue homeostasis

Cytokine alarmins:
subset of cytokines, including epithelial and myeloid cell-derived IL-25, TSLP, and IL-33. They are often released or secreted by stressed or damaged epithelial cells and can act to initiate and sustain innate and adaptive type 2 immune responses

Danger-associated molecular patterns (DAMPs):
also known as alarmins; endogenous molecules released from stressed/damaged cells that can promote immune cell activation

Efferocytosis:
the process where phagocytic cells engulf dead or dying cells

Neutrophil extracellular traps (NETs):
chromatin mesh that includes proteinases and peptidases capable of trapping and killing extracellular pathogens

Pathogen-associated molecular patterns (PAMPs):
conserved microbial structures that can activate innate responses

Pattern recognition receptors (PRRs):
receptors that recognize PAMPs and DAMPs, and primarily mediate the activation of innate immunity

Surfactant proteins:
innate immune molecules that are components of a surfactant and can contribute to pathogen resistance and modulating immune function

Trained innate immune response:
the conserved reprogramming of innate immune cells following stimulation, which results in an altered response after subsequent challenge

Tuft cells:
chemosensory epithelial cells lining the mucosal surfaces including the small intestine and lungs (brush cells)

Type 1 immune response:
triggered by certain microbial pathogens, resulting in the production of IFN-γ and other cytokines

Type 17 immune responses:
often triggered by extracellular pathogens and characterized by IL-17 production

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Box 1.

DAMPs/PAMPs of the Immune System

At least five different families of PRRs have been identified, Toll-like receptors (TLRs), composed of 13 different TLRs in both humans and mice, C-type lectin receptors such as Dectin 1-2, NOD-like receptors, RIG-I-like receptors, and AIM2-like receptors [4,112]. PAMPs binding TLRs can initiate type 1 and type 17 innate immune responses through the secretion of proinflammatory cytokines and chemokines, which provides adjuvant signals for Th1 and Th17 cell development. By contrast, specific PAMPs have not been identified as playing an essential role in initiating the type 2 immune response. Instead, the innate type 2 response appears more dependent on DAMPs, also known as alarmins, which are endogenous molecules often released/secreted by stressed, damaged, or dying cells. These DAMPs can include ATP, adenosine, and UA crystals. Many of these DAMPs, for example, ATP, may also play crucial roles in mediating various cellular functions during homeostasis. However, their release following cellular damage and their subsequent recognition by cell surface receptors are an essential mechanism used by the host to sense danger and activate the immune response. Along with TLRs, DAMP signaling can be mediated through the receptor for advanced glycation end-products, triggering receptors expressed on myeloid cells, and several ionotropic or G-protein-coupled receptors [113]. DAMPs can initiate innate immune responses through stimulation of non-immune cells and immune cells including macrophages, DCs, neutrophils, and mast cells, leading to the production of proinflammatory cytokines, recruitment of inflammatory cells, and activation of adaptive immunity [113,114].

Box 2.

Tuft Cells: Mucosal Tissue Guards

Tuft cells are taste-chemosensory epithelial cells present in small numbers in the small intestine, and in the lungs where they are also referred to as brush cells [115]. Recent studies have demonstrated a role for taste receptors expressed by these cells in orchestrating type 2 immune responses [116–118]. Tuft cells are the primary source of IL-25 in the gut, produced constitutively at readily detectable amounts [118,119]. During the early stages of *Hpb* infection in mice, tuft cell chemosensing of *Hpb* larvae leads to their secretion of **cysteinyl leukotrienes (cysLTs)**, which rapidly activate ILC2s in the presence of homeostatic IL-25 [120]. ILC2 activation and secretion of IL-13 induce goblet and tuft cell hyperplasia in mouse intestinal tract, leading to increases in IL-25, through a tuft cell IL-25-ILC2 circuit, thereby promoting a type 2 immune response and worm expulsion [116,118,119] (see Figure 1 in main text). Although ATP can activate airway tuft cells and induce production of cysLTs [121], it has not been shown to induce cysLT release in intestinal tuft cells in mice [120]; this suggests that another ligand might initiate cysLTs release, which remains to be tested. Tuft cells also express the taste-chemosensory succinate receptor (SUCNR1) in the small intestine where the microbial metabolite succinate secreted by *Tritrichomonas* protists binds SUCNR1 on tuft cells driving the tuft-ILC2 circuit and thereby promoting tuft cell hyperplasia and type 2 immune responses [122,123]. Although the metabolite succinate is secreted during helminth infection such as with *Nb*, the sensing of *Nb* is SUCNR1 independent, suggesting a redundant or a different signaling pathway being required for their activation during *Nb* infection [122]. Furthermore, both helminth-secreted excretory/secretory products and extract of *Ts* can stimulate another group of bitter-taste receptors (Tas2rs) on tuft cells enhancing the tuft-ILC2 circuit [124]. As the infection progresses, tuft cell expansion and secretion of IL-25 during the later effector phase of *Hpb* infection appear to be essential factors involved in worm clearance [117].

Outstanding Questions

How do specific recently identified factors and cell populations triggering type 2 immunity interact to orchestrate the overall response? Individual cell populations and molecules identified as essential may not be sufficient. Research is needed to reveal how these different factors together initiate the *in vivo* response.

What are the common and distinct features characterizing type 2 responses that develop in different TMEs and in response to different stimuli? Few studies have compared how type 2 responses develop and differ in response to distinct stimuli and microenvironments. Insights might reveal new candidate checkpoints that might be targeted to influence type 2 immunity initiation and progression.

How do cell death signaling pathways activated in response to tissue damage influence the initiation of type 2 responses? Given the importance of DAMPs in initiating type 2 immunity, understanding the nature of the cell death pathways involved might provide new insights into how to promote or control this response.

What is the role of helminth ES products in influencing the initiation of type 2 responses and associated immune regulatory circuits? ES products might provide an important source for the development of novel potential therapeutics to regulate specific components of harmful inflammation, or for the identification of new adjuvants to promote type 2 immunity.

Given the importance of ATP and its metabolites in initiating type 2 responses, what are the cellular sources of ATP and the mechanisms that regulate ATP and adenosine signaling? It will be important to tease apart the specific purinergic signaling pathways that contribute to the initiation of type 2 immunity, as well as how to potentially modulate its heterogeneity. These insights might provide a platform for the development of novel candidate treatments to treat a variety of type 2 immunity-related pathological responses.

Highlights

Danger associated molecular patterns (DAMPs) released in response to tissue damage are likely to play a major role in initiating type 2 responses in humans and mice.

The initiating factors of type 2 immunity can vary with the specific tissue microenvironment. Epithelial cells are of importance in barrier responses, while other cell types such as macrophages or stromal cells may be preferentially important at other tissue sites.

As the initiation of a type 2 immune response progresses, a variety of innate immune cells including macrophages and granulocytes, as well as innate lymphoid cells, are instrumental and often interact to shape the development of type 2 immunity.

In many cases, these initial interactions result in the production of cytokine alarmins, interleukin (IL)-33, IL-25, and thymic stromal lymphopoietin (TSLP), which then promote and amplify the type 2 immune response.

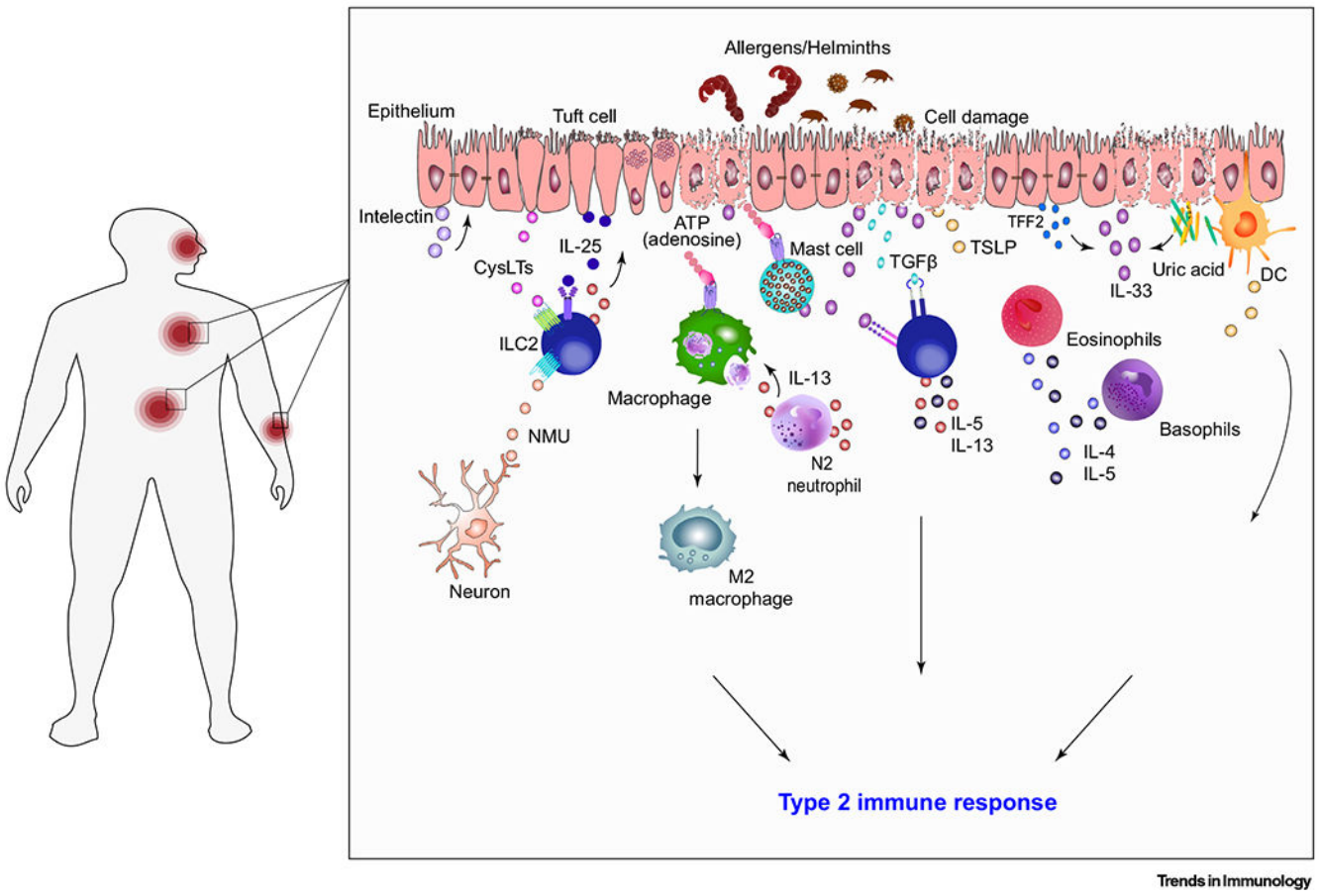

Figure 1. Helminths and Allergens Initiating Type 2 Immune Responses.

The schematic illustrates multiple often nonoverlapping pathways that together trigger type 2 immune responses in mice. DAMPs, such as extracellular ATP released from damaged cells, and the release of preformed IL-33, activate ILC2s and myeloid cells resulting in type 2 cytokine production, alternatively activated (M2) macrophages, and development of type 2 responses. Efferocytosis of neutrophils can also contribute to M2 macrophage activation [39]. Furthermore, damaged and dying cells secrete uric acid which binds and activates DCs, to initiate the type 2 immune response [125]. TGF-β released from protease damaged cells stimulates the production of IL-5 and IL-13 from ILC2s [126]. At early stages of infection, tuft cell chemosensing of larvae triggers the production of cysLTs, which activate ILC2s in the presence of constitutive IL-25 production by tuft cells [120]. Activated ILC2s produce IL-13, in turn leading to tuft cell hyperplasia and associated increases in IL-25 [116,118,119]. TFF2 secreted by epithelial cells stimulates the release of IL-33 and stimulates $CD4^+$ T cells to produce IL-4 and IL-13 [16]. Stimulation of epithelial cells by IL-13 also induces the production of intelectin which in turn increases the production of alarmins IL-25, IL-33, and TSLP. Although not shown, recent studies also suggest that IL-17 and type I interferons also contribute to the initiation of type 2 immunity [24,25,28]. Abbreviations: cysLT, cysteinyl leukotriene; DAMP, danger-associated molecular pattern; DC, dendritic cell; IL, interleukin; ILC2, group 2 innate lymphoid cell; N2, alternative

activated neutrophil; NMU, neuromedin U; TFF2, trefoil factor 2; TSLP, thymic stromal lymphopoietin; TGF-β, transforming growth factor-β.

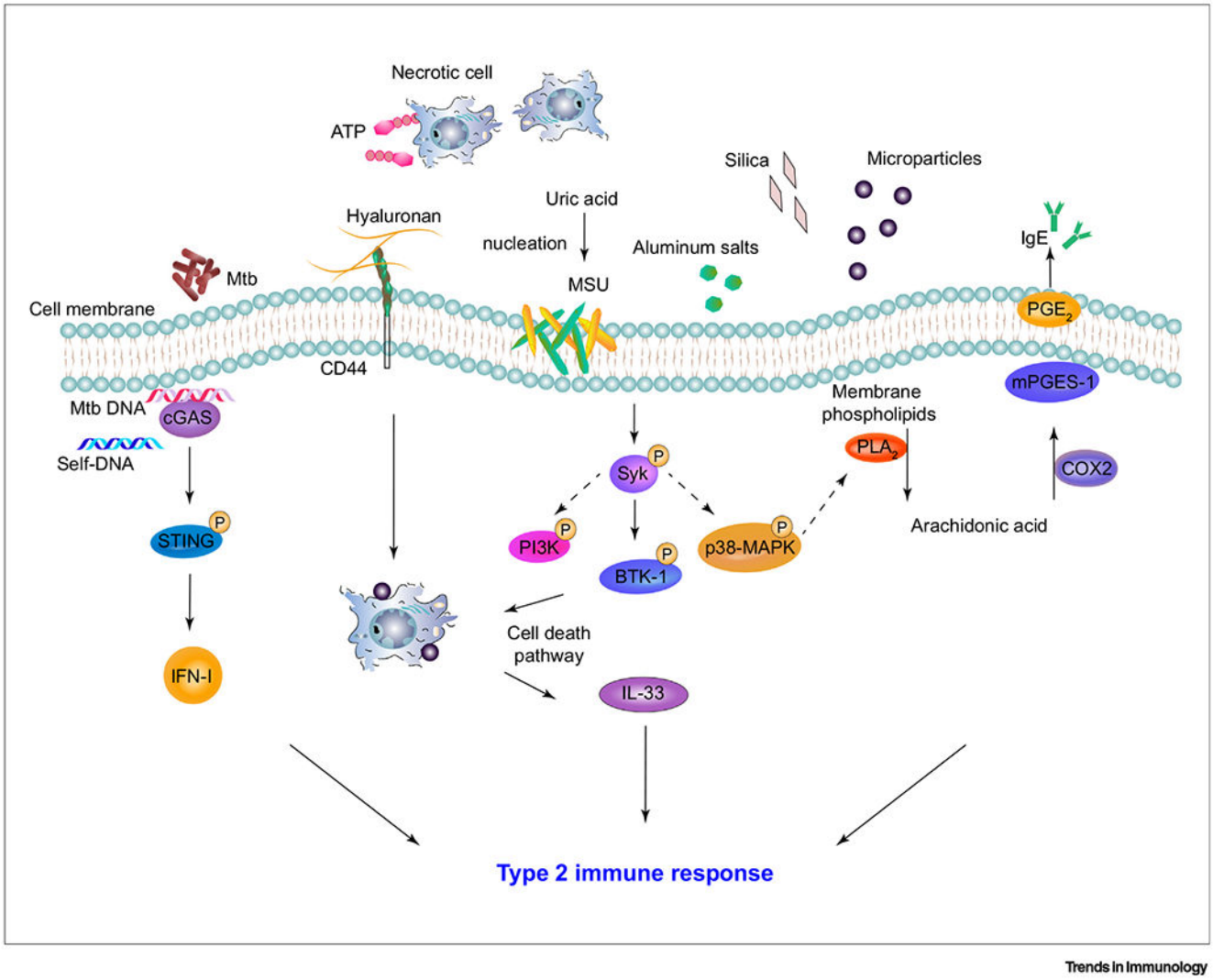

Figure 2. Signaling Molecules Initiating Type 2 Immune Response in Sterile Inflammation.

Microparticles, trauma, and tissue injury can trigger sterile inflammatory responses including innate type 2 immunity. In mouse models of liver injury, ATP recruitment of GATA-6^{+} macrophages and the activation via hyaluronan–CD44 can lead toM2 macrophage phenotype polarization [69]. Microparticles induce Syk and downstream BTK-1 signaling leading to the release of IL-33 in mice [62]. Silica has also been shown to prime type 2 immune responses by triggering the release of DNA by damaged cells, thereby activating the cGAS/STING pathway and increased IFN-I in mice [68]. Type 2 immunity is also mediated by activation of p38 MAPK and PLA_2, resulting in the release of arachidonic acid from membrane lipids. Arachidonic acid leads to the production of COX2 and mPGES-1, converting arachidonic acid to PGE_2, which can enhance IgE production in mice [54]. Abbreviations: BTK-1, Bruton's tyrosine kinase-1; cGAS, cyclic GMP-AMP synthase; COX2, cyclooxygenase-2; IFN-I, interferon-I; MAPK, mitogen-activated protein kinase; mPGES-1, membrane-associated PGE synthase-1; MSU, monosodium urate crystal; Mtb, *Mycobacterium tuberculosis*; PGE2, prostaglandin E2; PI3K, phosphoinositide 3-kinase;

PLA_2, phospholipase A2; STING, stimulator of interferon genes; Syk, spleen tyrosine kinase.

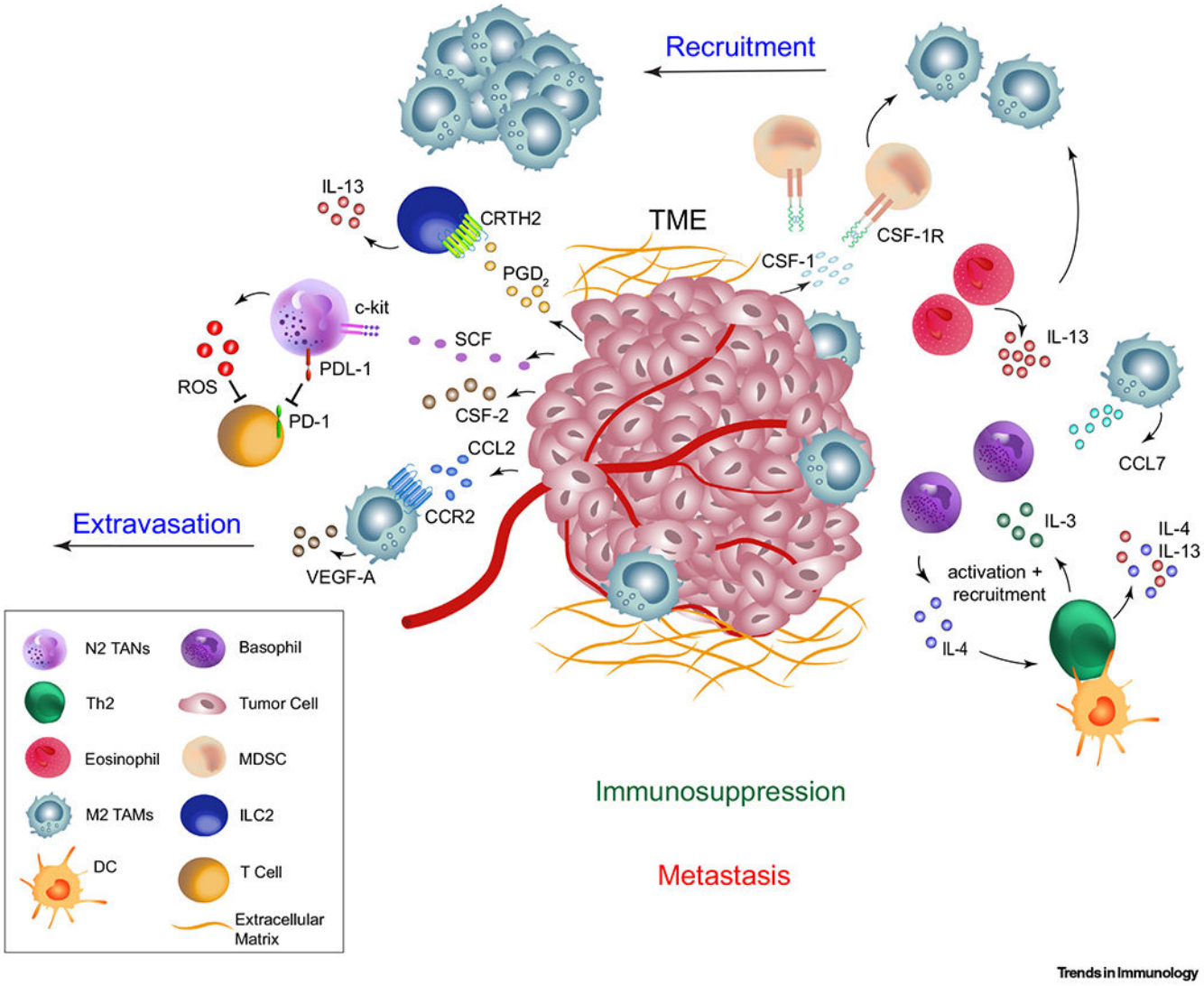

Figure 3. The Tumor Microenvironment Can Suppress Immune Responses by Initiating Type 2 Immunity.

Tumor cell secretion of PGD_2 stimulates CRTH2 signaling on ILC2s and their consequent IL-13 production and recruitment of MDSCs in mice [106,107]. Secreted CSF-1 can promote the differentiation of alternative activated (M2) macrophages, while CCL2 secreted from tumor and stromal cells and eosinophil secretion of IL-13 can promote M2 macrophage recruitment [95]. VEGF-A secreted by these M2 macrophages can lead to extravasation of tumor cells and metastasis in certain models [95–97]. Mouse M2 macrophage release of CCL7 can lead to the recruitment and activation of IL-4-producing basophils, in turn activating Th2 $CD4^+$ T cells to produce IL-4/IL-13 [110]. Furthermore, tumor-derived CSF-2 can activate N2 TANs and PD-L1 expression can suppress T cell function in certain APL and bladder cancer human patients [104]. Tumor derived SCF can also drive the production of ROS from TANs, leading to suppression of T cell function in mice [105]. Abbreviations: CCL, C-C motif chemokine ligand; CCR2, C-C chemokine receptor type 2; CRTH2, chemoattractant receptor-homologous molecule 2; CSF, colony-stimulating factor;

CSF-1R, CSF-1 receptor; DC, dendritic cell; ILC2, group 2 innate lymphoid cell; M2 TAM, M2 tumor-associated macrophage; MDSC, myeloid-derived suppressor cell; N2 TAN, N2 tumor-associated neutrophil; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PGD2, prostaglandin D2; ROS, reactive oxygen species; SCF, stem cell factor; Th2, T helper type 2; TME, tumor-microenvironment; VEGF-A, vascular endothelial growth factor A.