Regulation of type 2 immunity to helminths by mast cells

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Recently, we demonstrated a novel role for gastrointestinal mast cells (MCs) in the early events that lead to the generation of Th2 immunity to helminth infection.1 Mice lacking MCs (KitW−/− and KitW−Sh) showed a significant inhibition of Th2 cell priming following infection with the parasitic helminth Heligmosomoides polygyrus bakeri (Hp). We showed that MCs degranulate during the early stages of infection when the helminth larvae invade the small intestinal tissue. Furthermore, MC degranulation was required for the enhanced expression and n of the tissue-derived cytokines IL-25, IL-33 and TSLP, which are required for the optimal orchestration and priming of type 2 immunity. In this addendum we aim to address several questions raised by our findings — in particular, the mechanisms through which MCs may recognize helminth exposure in the early stages of infection and by which they may enhance expression of critical tissue cytokines thus, enabling Th2 priming. Furthermore, we will discuss these findings in the context of recently described novel innate immune cells, such as type 2 hematopoietic progenitors and type 2 innate lymphoid cells.

Introduction

Infections with gastrointestinal helminths remain highly prevalent, particularly in developing countries, with over 1 billion humans estimated to be infected worldwide.2 Helminth infection results in nutrient malabsorption, intestinal inflammation, impaired growth and development and reduced educational performance in the host.2 Conversely, helminth infections have been shown to play key roles in the suppression and modulation of inappropriate inflammation, and loss of helminths as a result of increased hygiene or drug treatments results in an increased rate of autoimmune, inflammatory and allergic disorders (reviewed in ref. 3). Furthermore, experimental treatments with live helminths are currently undergoing clinical trials to test their efficacy as novel therapeutics in a variety of human disease settings.3 Thus, understanding the pathways that generate, modulate and regulate the host immune response during helminth infection is of significant clinical relevance.

Mast cells (MCs) are a potent arm of the innate immune system and can be found in barrier tissues throughout the body. MCs are induced by cytokines such as stem cell factor (SCF), IL-3, IL-4 and IL-9 and accumulate in the inflamed tissue. In response to a wide range of infections MCs rapidly release a multitude of inflammatory mediators including histamine, proteases and cytokines (reviewed in ref. 4). During gastrointestinal helminth infections MCs are traditionally considered a key effector cell during late stage T helper 2 (Th2) associated inflammation, where they cross-link antigen specific IgE via the surface high affinity FcεRI and degranulate. The effector role for MCs during helminth infection has been shown to be important for the expulsion of many helminth species from the gastrointestinal tract (reviewed in ref. 5). In contrast, the innate IgE-independent role of tissue resident MCs during the early stages of helminth exposure is relatively poorly understood. MCs possess a wide range

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Abbreviations: MC, mast cell; Hp, Heligmosomoides polygyrus bakeri; iLC, innate lymphoid cell; lin, lineage marker cocktail

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of receptors that enable them to innately sense pathogen associated molecules and to respond rapidly. Recent studies have suggested that tissue resident MCs provide critical adjuvant signals following antigen exposure that amplifies the priming of the adaptive immune response. In particular, rapid MC degranulation and histamine release immediately following allergen exposure was required for the induction of early inflammation and migration of dendritic cells to the lymph node to prime adaptive immune responses in a model of contact hypersensitivity.

Similarly, in our recent study we demonstrated that within the first days of \( H_p \) infection in the small intestine MCs degranulate in an IgE-independent manner and that MC mediator release was required for the upregulation of tissue-derived cytokines (IL-25, IL-33 and TSLP) and optimal migration of dendritic cells required for Th2 cell priming. However, to date the mechanisms through which MCs sense worm infection and the specific mediators released by MCs that mediate enhanced orchestration of tissue cytokine production and type 2 immunity are unknown. Below, we speculate on some of the candidate pathways through which MCs may mediate their effects during gastrointestinal helminth infection.

### How Do Mast Cells Sense Gastrointestinal Helminth Infection?

MCs, like many cells of the innate immune system, are equipped with a wide range of pathogen sensing receptors that allow them to recognize danger and are often referred to as “sentinels” of the immune system (Fig. 1; left panel). In our recent study we showed that MCs degranulate within the first days of a helminth infection in an IgE-independent manner — but the way in which MCs recognize intestinal worm infections are unknown. Attempts to delineate these pathways are complicated because helminth infections in the gastrointestinal mucosa also inevitably lead to exposure of intestinal MCs to signals derived from the abundant commensal bacteria. As such, we hypothesize that MCs may (1) recognize helminth derived products directly; (2) recognize invading commensal bacteria signals with concurrent bystander effects on the anti-helminth response; (3) require dual signals from both commensals and helminths. In addition to pathogen derived signals, infection also leads to significant tissue damage and thus, intestinal MCs are also likely to be exposed to a range of danger signals.

Helminth excretory/secretory (E/S) products represent an abundant source of stimulatory molecules that can be recognized by MCs. For example, it was recently shown that \( H_p \) E/S is dominated by a group of venom-allergen like proteins (VAL) and similar proteins are found to be produced by a wide range of parasitic worms. Interestingly, one of the main roles of MCs in barrier tissues has been shown to be to recognize venom proteins from pathogens and to release mediators that inhibit their toxicity to the host.
How Do Mast Cells Contribute to Enhanced Tissue-Derived Cytokine Production?

Tissue-derived cytokines such as IL-25, IL-33 and TSLP have been shown to have essential roles during helmint infection, and mice lacking any of the three cytokines show abrogated priming of Th2 cells and reduced anti-parasitic responses. MCs express the IL-33R (ST-2 + IL-1RAcP) and become activated upon IL-33 stimulation resulting in the release of inflammatory mediators. Thus, one possibility to be explored is that MCs secrete signals derived from tissue damage such as IL-33 during the initial stages of helmint invasion and enhance tissue cytokine expression, including IL-33 itself, via a novel positive feedback mechanism.
Control of Type 2 Innate Populations Via a Mast Cell-IL-25/IL-33 Axis?

Fully differentiated mature MCs are only found in the tissues that are populated by bone marrow derived populations of MC-precursors that circulate in the blood. The exact signals that attract MC-precursors to the tissues and stimulate them to undergo differentiation are not fully elucidated, although SCF and IL-3 are known to play critical roles. As detailed above we identified a role for MCs in regulating the tissue-derived cytokines IL-25 and IL-33 in the intestine during the early stages of helminth infection. These cytokines have been shown to play critical roles in orchestrating innate type 2 immunity, including the recruitment of Lin- progenitors with the capacity to differentiate to MCs. In our recent study we observed the expansion of a Lin- cell population in the small intestine during Hp infection in WT mice, but not MC deficient mice, that expressed the hematopoietic markers CD34 and Sca-1. When these cells were isolated and cultured with SCF and IL-3 they upregulated the expression of the c-kit and FceRI, indicative of a MC phenotype. In line with previous reports these progenitors could be partially restored in MC deficient mice (that lack tissue IL-25 following infection) upon treatment with exogenous rIL-25. Thus, this leads to the intriguing hypothesis that MC degranulation following infection plays an important role in repopulating barrier tissues with MC progenitors by the regulation of an IL-25 driven axis.

In addition, many recent studies have identified populations of type 2 innate lymphoid cells (iLCs) in the lymph node and tissues that produce IL-13 during helminth infection and allergy. Expansion of these cell types is also critically regulated by IL-25 and/or IL-33 production in the inflamed tissue and these cells have all been characterized by their lack of conventional immune lineage markers (Lin-), as well as their expression of a range of other surface markers including IL-33R (ST-2), the IL-7Rα subunit (CD127), the activation molecule ICOS and intermediate expression of c-kit, among others. In several independent studies a range of similar cell types were identified and differentially termed Natural Helper Cells (NHC), Nuocytes or Innate helper 2 cells. Collectively these cells are referred to as type 2 iLCs as they share the ability to produce type 2 cytokines but also share similarities with pro-inflammatory innate lymphoid lineages that produce IFN-γ, IL-17A and IL-22.

Given the expression of IL-25 and IL-33 following Hp infection we hypothesized that type 2 iLCs may expand in the draining lymph node during the early stages of infection. Administration of exogenous rIL-25 to Hp infected mice during the first four days of Hp infection elicited a population of Lin- cells in the MLN, whereas only a negligible increase in the same cells could be seen during Hp infection alone in WT mice compared with naive WT mice and MC deficient mice that lack upregulated expression of IL-25 and IL-33 (Fig. 2A). rIL-25 induced Lin- cells expressed ST-2, CD127, ICOS and were c-kit+ in line with previous descriptions of type 2 innate lymphoid cells (Fig. 2A and B). The population resembling type 2 iLCs were also found to express high levels of IL-13, in contrast to other Lin- cells in the lymph node (Fig. 2C), suggesting these cells truly resemble type 2 iLCs. Interestingly, we reported that administration of exogenous IL-25 in WT mice during Hp infection resulted in significant worm expulsion in this otherwise chronic model of helminth infection. Thus, it is possible that high numbers of type 2 iLCs can drive expulsion during Hp infection as reported in other infections such as Nippostrongylus brasiliensis.

Thus, it is not yet clear why we could detect a significant induction of Lin- MC progenitors in Hp infected WT mice that expressed both endogenous IL-25 and IL-33 but only a small induction of type 2 iLC cells. One possibility is that a higher threshold of IL-25 expression is required for the induction of type 2 iLC populations than is needed for the recruitment of the MC-progenitor population. Indeed the concentrations of exogenous rIL-25 that were systemically administered are fairly high (0.4μg/injection) and it is not yet clear if this truly resembles physiological expression levels. Another possibility is that the action of endogenous IL-25 and IL-33 elicited during Hp infection is restricted to the tissues and has only limited systemic effects. Alternatively, induction of type 2 iLCs may be selectively dependent on other cellular sources of IL-25 and IL-33 outside of the inflamed tissue. For example, radio-resistant cells in the spleen can produce IL-33 following viral infection in the lung. Furthermore, as reconstitution of MC deficient mice with IL-25 also restored the expression of IL-33 and TSLP, we cannot rule out that the recruitment of such progenitor is not directly dependent upon IL-25 itself, in contrast to type 2 iLCs, and rather on the downstream induction of other signals. Further work is needed to delineate the pathways that orchestrate these responses in the tissues and periphery following helminth infections.

Impact and Outlook

MCs are distributed throughout barrier tissues and are highly immunocompetent cells that express the required machinery to sense pathogenic stimuli and to rapidly respond via release of a multitude of inflammatory mediators. MCs have been described to have key early roles in the induction and amplification of immune responses in a wide range of diseases and increasing evidence suggests one way in which they contribute to this process is via the regulation of tissue-derived cytokines such as IL-25, IL-33 and TSLP. The precise mechanisms through which MCs may fulfill this function during intestinal helminth infections are yet to be elucidated. Future
Type 2 innate lymphoid cells are rare in the ML during the early stages of Hp infection but can be induced by exogenous rI-25 treatment. C57BL/6 mice infected with 200 Hp L3 and Mesenteric Lymph Node (MLN) derived lymphocytes isolated and stained for a population resembling type 2 innate lymphoid cells via flow cytometry. A) Total numbers of type 2 innate lymphoid cells were quantified at day 6 post infection (p.i.) in the MLN of WT (black bars) and mast cell deficient Kit<sup>−/−</sup>/Kit<sup>Wt/Wt</sup> mice (white bars) following Hp infection alone or Hp infection with rI-25 treatment (0.4 μg i.p. on days 0–4 p.i.). B) Cells were gated as low/negative for a lineage cocktail (CD3/CD4/CD19/CD11b/CD11c) (shaded gate) and expressed the surface markers ST-2 (IL-33R), CD127 (IL-7Ra), ICOS and were c-kit low/int. C) Expression of IL-13 in Lineage low/negative gated ST-2+ or ST-2- populations in Hp infected, rI-25 treated mice. Data are representative of two independent experiments with n = 4–6 mice per group.

Figure 2.

work should focus on dissecting the contribution of MCs as sensors and amplifiers during the early stages of tissue immune responses. Recently developed inducible MC specific knockout mice should allow for significant advances in this field.

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