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Deciphering the Role of Eosinophils in Solid Organ Transplantation

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Abstract

Eosinophils are rare granulocytes that belong to the innate arm of the immune system. This cell population is traditionally defined as a destructive and cytotoxic mediator in asthma and helminth infection. Limited data in transplantation has suggested that eosinophils play a similar role in potentiating deleterious organ inflammation and immunologic rejection. Contrary to this long-held notion recent data has uncovered the possibility that eosinophils play an alternative role in immune homeostasis, defense against a wide range of pathogens, as well as downregulation of deleterious inflammation. Specifically, translational data from small animal models of lung transplantation has demonstrated a critical role for eosinophils in the downregulation of alloimmunity. These findings shed new light on the unique immunologic features of the lung allograft and demonstrate that environmental polarization may alter the phenotype and function of leukocyte populations previously thought to be static in nature. In this review we provide an update on eosinophils in the homeostasis of the lung as well as other solid organs.

Origin of the Eosinophil

Eosinophils are granulocytes that develop in the bone marrow from common myeloid progenitors (human[1]) or granulocytic/macrophage progenitors (mouse[2]) into eosinophil lineage-committed progenitors[3]. Transcription factors GATA-1 and Xbp1, along with several others, coordinate the differentiation of these $CD34^{+}$IL-5Rα^{+}progenitors into fully differentiated eosinophils ($CD34^{-}$IL-5R$\alpha^{+}CCR3^{+}$Siglec-F^{+} or Siglec-8^{+} human) in the bone marrow[4]. Differentiation and survival of eosinophils in humans and mice is highly dependent IL-5 and signaling through the IL-5Rα while their migration to other tissues is orchestrated by a group of chemokines known as eotaxins that bind the chemokine receptor 3 (CCR3)[5]. At homeostasis, once eosinophils exit the bone marrow, they have a half-life of ~1.8 days in the blood and migrate into the thymus, secondary lymphatics, adipose tissue,

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gastrointestinal tract (except esophagus), lung, skin, and uterus in both mice and man. Albeit their function in these locations are not fully defined[3,6] and their half-life varies greatly per tissue. Recent studies indicate that eosinophils contribute to developmental organ remodeling, metabolic homeostasis, microbiome homeostasis, and act as sentinels for infection and cancer[6–10].

Eosinophils in Asthma

The potential role of eosinophils in solid organ transplants may be inferred from a deeper understanding of allergic asthma. As a disease allergic asthma is defined by reversible airways hyperreactivity that is often associated with an increased mobilization of eosinophils into the lungs[11–13]. Increased absolute blood eosinophil count, sputum count of eosinophils >2–3% or presence of extensive eosinophil degranulation in lung biopsies are measures to define eosinophilic asthma and associate with disease severity[11,13]. Various stimuli control the generation, differentiation, migration and activation of eosinophils in allergic inflammation and asthma, including the Th2 associated cytokines IL-3, IL-4, IL-5, IL-13, IL-33 and GM-CSF, eotaxins, IgA and IgG, PAMPs and DAMPs; secreted by a wide variety of cells. To reduce the numbers of lung eosinophils in asthma, and thus their classic role as destructive cells[14,15], many newly approved biologics are aimed at inhibiting eosinophil survival and recruitment pathways (e.g., IL-5/IL-5Ra or CCR3)[16,17]. Although eosinophil degranulation is considered a paradigm of noxious response by these cells that leads to pathology in asthma, the secondary granule proteins from eosinophils, eosinophil peroxidase (EPX), eosinophil cationic protein (ECP), major basic protein-1(MBP-1), and eosinophil derived neurotoxin (EDN) are recently being found to have immune modulating functions[18]. Moreover, eosinophils have the capacity for specific and targeted release of immune mediators from their secondary granules, such as IL-4, through piecemeal degranulation or release of microvesicles[6]. In agreement with this, in mouse models of severe asthma with extensive eosinophil degranulation, deficiencies in EPX or MBP-1 was insufficient to reduce pathology, while deficiencies in eosinophil-derived IL-13 blocked mucus production in the airways and airways hyperresponsiveness to methacholine challenge[19]. These studies exemplify previously underappreciated roles for eosinophils as not just destructive mediators, but cells that participate in the immune responses in the lung.

Eosinophils in infectious disease

Eosinophils modulate immunity against various infectious pathogens. These include infection with helminth[20], nematode microfilariae[21,22], viral infection[23–25], and infection with bacteria[26–28]. The protective impact of eosinophils against infectious agents varies depending on the stage of infection and the type of infectious agent to include either killing of the agent or limitation of growth/symbiotic relationship. For example, eosinophil exposure to *Clostridium rodentium* induced killing through formation of eosinophil extracellular DNA traps and yet eosinophil exposure to *Helicobacter pylori* triggered immune mechanisms that permitted bacterial survival in the host[28]. In general direct killing mechanisms include release of granule proteins, enzymes and release of DNA extracellular traps[29]. Eosinophils are thus critical for IL-25 and IL-33/ILC-2 mediated protection against *Clostridium difficile* infection[26,30] and the virulence capacity of *C. difficile* was enhanced

under conditions that suppressed colonic eosinophilia[26,27]. Destruction of viruses by eosinophils occurs due to a mixed immune response. For example, influenza A and parainfluenza are targeted by Th1 activated and eosinophil-dependent effector functions to mediate iNOS-mediated killing of viruses, MHC class I antigen presentation of viral proteins and recruitment of $CD8^+$ T cells[23,24]. Strikingly the induced accumulation of eosinophils in allergic asthma models has been shown to dramatically improve viral titer loads and improve viral killing in mice[31,32]. Together, these studies demonstrate eosinophils have protective functions in complex immune responses and perform their protective activities through a wide variety of mechanisms.

Eosinophils in Cancer

As in other immunological disease settings, the role of eosinophils in cancer is diverse and microenvironment dependent[33–35]. Eosinophils have been identified in several cancers of both epithelial and non-epithelial origin, including pancreatic cancer, glioblastoma, bladder, cervical, colorectal cancer, as well as melanoma, and lymphoma. The presence of tumor associated tissue eosinophils as a prognostic indicator of disease outcome varies significantly as eosinophils have been shown to have both pro- or anti-tumorigenic functions. In part this is likely due to the stage and type of cancer as well as the local microenvironment. For example in oral squamous cell carcinoma it has been proposed that early stage carcinoma, which is a Th1-type microenvironment, leads to anti-tumorigenic activation of eosinophils while later stages of carcinoma develop a Th2-type microenvironment and promote pro-tumorigenic potential of eosinophils[36,37]. In mouse models of colorectal cancer, where IFN-γ is increased, eosinophils directly kill tumors independent of $CD8^+$ T cell functions[38]. This supports findings in colorectal patients where eosinophils correlate with better prognosis and fewer metastasis[39]. Alternatively in melanoma $CD8^+$ T cell recruitment is enhanced by INF-γ and TNF-α activated eosinophils, resulting in melanoma tumor killing[40]. Thus in a complex disease such as cancer the role of the eosinophil varies based on the tumor microenvironment.

Eosinophils in immunosuppression

Despite their established role in cytotoxicity we, as well as others, have previously proposed that eosinophils have the capacity for broad dynamic differential activation and may vary their function drastically based on environmental cues[9,41]. The mechanisms of immune suppression by eosinophils that have been identified primarily in animal models are through either release of suppressive molecules and/or cell interactions with dendritic cells and lymphocytes. A homeostatic mechanism of immune suppression has been identified for tissue resident eosinophils in the lung that exhibit suppressive capacity to allergen induced Th2 immune responses[42]. Here, eosinophils mediate suppression through inhibition of dendritic cell activation, reducing activation of Th2 cells to allergen exposure in the airways. Conversely, we have shown that recruited eosinophils to the lung mediate suppression of Th1/Th17 cells through dendritic cells in allergen models of asthma[10,43]. The mechanistic diversity of eosinophil mediated immune suppression were further demonstrated in humans where a subset of $CD16^{hi}$ eosinophils were described to have suppressive capacity against T cells through a mechanism that is partially dependent on their upregulation of galectin-10

induced synapse formation[44]. Similar functions have been suggested in multiple myeloma[45]. In homeostatic conditions, eosinophil-T cell suppression has been demonstrated in the thymus, where eosinophils release indoleamine 2 3-dioxygenase to suppress Th1 polarization during thymic development[46].

Eosinophils in solid organ transplantation

To date the role of eosinophils in organ transplantation remains poorly explored leading to confusion and controversy in the field. Early data generated in the 1980s suggested that eosinophils solely contribute to the rejection of most solid organs[47–49]. This was based on early studies associating peripheral blood eosinophilia to episodes of graft rejection[50,51] as well as correlative studies linking tissue eosinophilia to graft rejection[47,49,52,53]. Recent studies continue these correlations, proposing eosinophil blood counts as a biomarker for acute liver rejection[54,55]. Yet a recent meta-analysis of >800 patients and more than 1000 sample points indicated that blood eosinophil counts are highly heterogenous and have only a 50% sensitivity for predicting acute liver rejection[56]. Despite the fact that limited mechanistic evidence was provided by any of these studies the destructive role of eosinophils was inferred from their increase at the time of rejection. In addition the predominance of eosinophil accumulation in the setting of irreversible graft dysfunction, compared with reversible graft damage, was used to further justify the role of eosinophils as mediators of graft rejection[48,49]. In many regards the view of eosinophils in graft rejection were similar to those of eosinophils in asthma; a sign and possible mediator of pathological inflammation and disease.

Eosinophils have been shown to influence Th2 polarization of the microenvironment[57,58]. Traditional data has suggested that Th1 polarization of the graft environment results in graft rejection while Th2 polarization results in tolerance and amelioration of the rejection response. For example pediatric liver allograft recipients with a predominantly a Th2 polarized cytokine profile have significantly reduced rates of rejection compared to patients with a Th1 cytokine profile[59]. Similarly, in renal transplant recipients it is clear that T cell clones from patients with chronic graft rejection produce higher levels of the Th1 associated cytokine IFN-γ, while T cells from accepting patients produce higher levels of IL-10[60]. Nevertheless such a seemingly clear cut notion is confounded by the demonstrations that adoptive transfer of Th2 polarized T cells can mediate graft rejection of both skin and heart grafts in $Rag^{-/-}$ or SCID mice even in the absence of classic cytotoxicity *in vitro*[61–63]. In addition Th2 polarized graft rejection demonstrates severe infiltration of eosinophils[64]. Taken together such data suggests that eosinophils, and their Th2 polarizing environmental influence, could play a role in the rejection of tissues and organs.

Conventional methods of achieving eosinophils depletion, such as antibody mediated blockade of IL-5, failed to ameliorate the rejection of pig pancreatic proislets transplanted to mice, notwithstanding the effectiveness of the treatment in drastically reducing eosinophil infiltration into the xenografts[65]. This finding led the authors to question the clinical practice of using the infiltration of eosinophils into graft biopsies as a prognostic indicator for graft rejection and highlighted the need for a better definition and understanding of the role of eosinophils in graft tolerance and rejection. These findings led to the speculation that

eosinophils might not primarily contribute to graft rejection but instead may play a secondary or accessory role in this process[65]. Alternatively it was proposed that eosinophils might essentially be innocent bystanders in some transplant models[47]. This notion was further supported in a model of allogeneic cardiac transplantation, where eosinophil depletion had a very minor impact on the survival of fully MHC mismatched heart allografts in the absence of $CD8^+$ T-cells[66]. This data suggested no role or a CD8+ T cell-dependent role for eosinophil-mediated immunoregulation.

Additional confusion regarding the role of eosinophils in cardiac graft rejection arose from recent studies demonstrating that acute cellular rejection, as well as antibody mediated rejection, was associated with low level of blood eosinophils[67]. These authors put forth a possibility that an immunologic state of quiescence, manifested by a higher eosinophil counts, maybe involved in preventing immunologic graft rejection[67]. This study therefore opened the possibility that eosinophils might be playing a role in transplantation tolerance and may not unequivocally contribute to graft rejection. Nevertheless low numbers of eosinophils in most solid organs, such as hearts kidneys and livers, makes their role difficult to decipher.

Eosinophils in lung transplantation

Unlike most transplantable solid organs lung alloimmune responses occur within the graft independent of secondary lymphoid tissue[68–70]. Lungs also contain a large population of eosinophils, making them and ideal organ to investigate the role of this granulocyte in the alloimmune response. Observational data has been interpreted to suggest that accumulation of tissue eosinophils is associated with acute lung allograft rejection. Such a notion is based on their presence in bronchoalveolar lavage fluid (BAL) during acute rejection episodes. Nevertheless the significance of such eosinophilia in pathobiology of lung allograft rejection was not well defined and unclear[52,53,71].

In order to establish clarity regarding the role of eosinophils in lung allograft homeostasis we utilized a clinically relevant murine model of left lung transplantation[72,73]. We initially depleted eosinophils from lung graft recipients through either neutralization of IL-5 or targeted deletion in transgenic mice, where the diphtheria-toxin receptor is expressed under the control of eosinophil peroxidase promoter (iPHIL mice)[74,75]. Interestingly eosinophil depletion did not ameliorate rejection but actually prevented co-stimulatory blockade (CSB)-mediated graft acceptance and accentuated rejection in the absence of immunosuppression (Figure 1). Unlike previous reports, that demonstrated an association of Th2 cytokines with the tolerance[76–78], we demonstrated that both CSB-treated accepting as well non-immunosuppressed rejecting grafts demonstrate a Th1 polarized microenvironment[74,75]. This was marked by increased expression of IFN-γ and TNF-α without significant impact on Th2-associated cytokines such as IL-4, GM-CSF and IL-33[74]. As suspected more pronounced Th1 polarization was evident in rejecting lungs that were not treated with immunosuppression but even in the presence of CSB, IFN-γ and TNF-α -mediated responses predominated[74,75]. Our observation supports the unique role of pro-inflammatory mediators in promoting lung allograft tolerance[79] and reaffirms the uniqueness of lung-specific immune responses[68,70,80–82]. In line with Th1 polarization of the lung allograft

microenvironment, eosinophils from both accepting and rejecting lung grafts were polarized to a Th1 (or E1)-like phenotype[74,75]. The Th1 signature of the lung allograft eosinophil differs from the Th2 (or E2)-type associated with asthma and allergic inflammation[57].

We explored the regulatory capacity of such Th1 polarized eosinophils. Indeed Th1 polarization was a key component to eosinophil regulatory capacity as amelioration of the Th1 phenotype blocked eosinophil-mediated suppression[74]. Interestingly we and others have previously described the expression of inducible nitric oxide synthase (iNOS) to be a key component of lung-specific immunoregulation[74,83,84]. Th1 polarization was characterized by upregulation of iNOS in lung resident eosinophils. In fact eosinophils were the dominant and sole producers of iNOS in the lung graft[74]. To this end we uncovered that the capacity of the Th1 polarized eosinophils to induce T cell mediated immunosuppression was dependent on their ability to upregulate iNOS and form a synapse with $CD8^+$ T cells through PD-1/PD-L1 interactions[75]. Since PD-L1 expression is also upregulated by Th1 cytokines[85] we therefore proposed a unique and possibly lung allograft-specific feedback loop whereby CD8+ T cells produce IFN-γ that drives eosinophils to express PD-L1 and iNOS which leads to synapse formation with CD8+ T cells to prevent effector differentiation (Figure 2). Depletion or neutralization of any component in this feedback loop prevents lung graft acceptance[74,75,82]. A similar mechanism for the downregulation of IFN-γ dependent Th1 immunity and eosinophil-T cell interactions has been reported in a gastrointestinal bacterial infection model[28], as well as graft vs. host disease[44], and in the downregulation of anti-tumor immunity against multiple myeloma[45]. Importantly, in the gastrointestinal bacterial infection model it was shown that the eosinophil dependent feedback inhibition of T cell mediated Th1 responses is only partially dependent on PD-L1. This reinforces our observation that in the lung PD-L1 is only necessary for the establishment of contact and immunological synapse between the eosinophil and CD8+ T cell while the amelioration of T cell activation and alloimmunity is iNOS dependent[28,75].

Despite this many questions remain. While we have provided strong data regarding the supporting role of eosinophils for initial graft acceptance, their role in chronic graft fibrosis is unknown. Eosinophils were identified in 14 out of 15 examined allograft nephrectomies performed for obliterative arteriopathy. In the same report eosinophil conditioned media stimulated smooth muscle proliferation suggesting a direct link between eosinophil infiltration and chronic vascular rejection[86]. In lung allograft recipients increased BAL and blood eosinophilia were associated with worse graft outcome and chronic lung allograft dysfunction (CLAD). In fact BAL eosinophilia has been used to predict a form of graft fibrosis defined as restrictive CLAD (rCLAD)[71,87]. In murine models the role of eosinophil-mediated fibrosis has been linked to their promotion of collagen expression in epithelial cells through production of TGF-β after therapeutic radiation in the gut, another mucosal barrier organ[88]. In asthma models eosinophils were demonstrated as dispensable for airway hyperresponsiveness and mucous secretion, but critical for peri-bronchial collagen deposition[89]. Thus our recent proposal to facilitate lung allograft acceptance through increased eosinophil migration into the lung[75] might come with a price of long-term CLAD. These and other aspects of eosinophil biology surrounding lung transplantation are thus prime areas for investigation.

Summary

While traditional dogma suggests that eosinophils are uniquely destructive in their actions, and contribute solely to allograft rejection, our recently expanded understanding of their biology puts this notion into question. It is now evident that eosinophils are uniquely suited to exert immunomodulatory functions largely influenced by their microenvironment. While their role in lung organ transplantation is still unclear, recent work from our laboratory has demonstrated that under Th1 pro-inflammatory conditions eosinophil polarization plays a critical part in a feedback loop that controls excessive inflammation and tissue damage as well as tolerance. In certain Th1 environments eosinophils maintain the moderate inflammatory state required for immunosuppression-mediated tolerance, but may also enhance the survival of some pathogens and aggravate infections. Conversely in other contexts eosinophil Th1 polarization may induce pathogen killing. This recent understanding of the immunomodulatory role of eosinophils in the lung and gastrointestinal track situates eosinophils as potential guardians whose efforts to protect their host tissues could sometimes be excessive and lead to detrimental side effects. Nevertheless the potential for manipulating the eosinophil to facilitate organ specific tolerance offers exciting and novel avenues of translational investigation and deserves further work.

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Abbreviations:

iPHIL mice	mice expressing diphtheria-toxin receptor under the control of eosinophil peroxidase promoter
CLAD	chronic lung allograft dysfunction
iNOS	inducible nitric oxide synthase
CSB	co-stimulatory blockade
PAMPs	pathogen associated molecular patterns
DAMPs	danger associated molecular patterns
BAL	bronchioalveolar lavage

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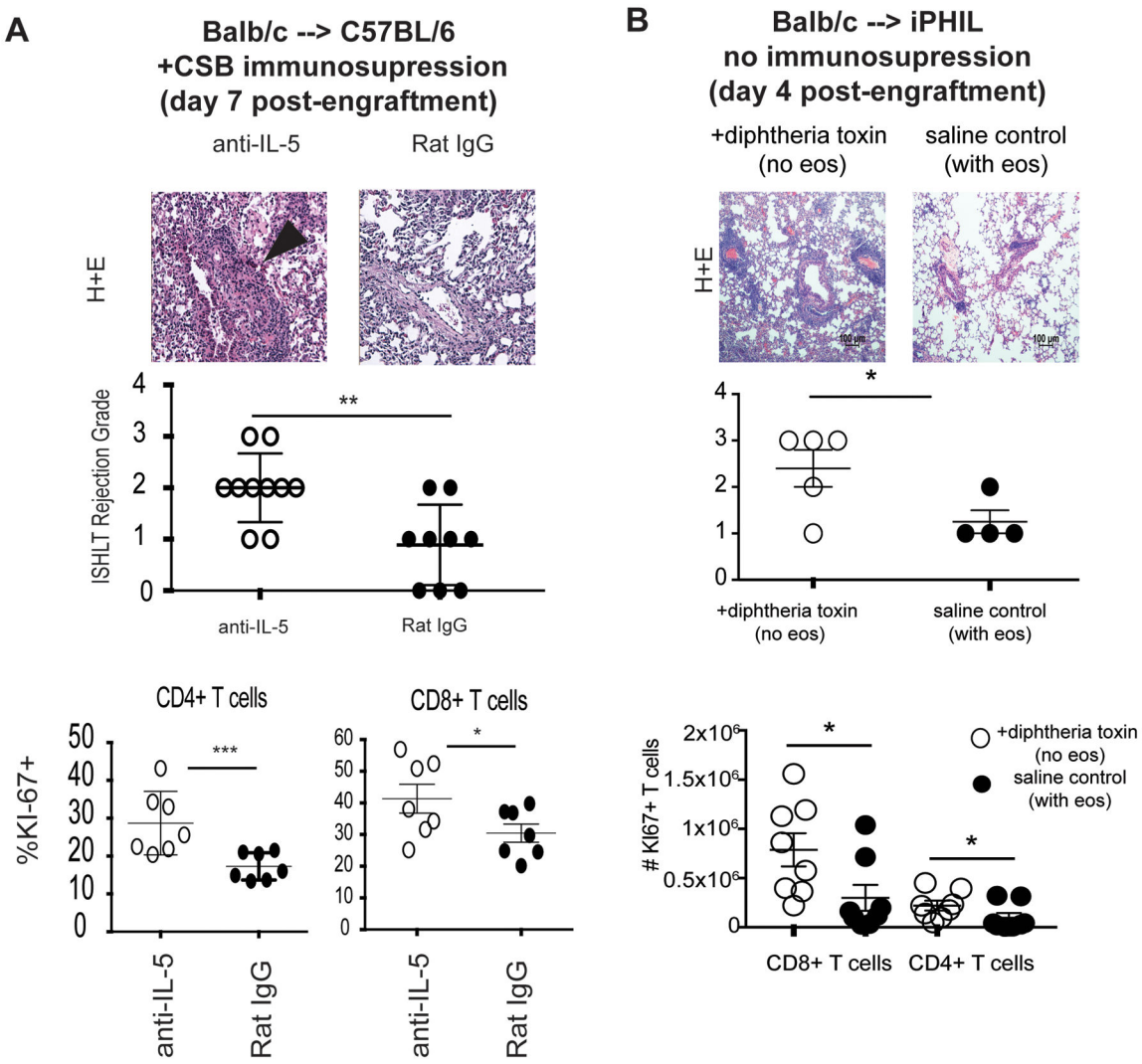

Figure 1. Eosinophil mediated suppression of T cell alloimmune responses

(A) ISHLT A rejection grade and KI67 (expressed as %) in CD8+ and CD4+ graft resident T cells seven days after engraftment of Balb/c lungs to C57BL/6 recipients with eosinophil depletion accomplished by IL-5 neutralization. All grafts were treated with co-stimulatory blockade immunosuppression affected by CTLA-4 Ig and anti-CD40L (clone MR1). (B) ISHLT A rejection grade and T cell proliferation (expressed as total number of KI67+ T cells) in Balb/c lungs engrafted to iPHIL mice on a C57BL/6 background with eosinophil depletion accomplished by diphtheria toxin administration. (reproduced from[74,75] *** p<.001, **p<.01, *p<.05)

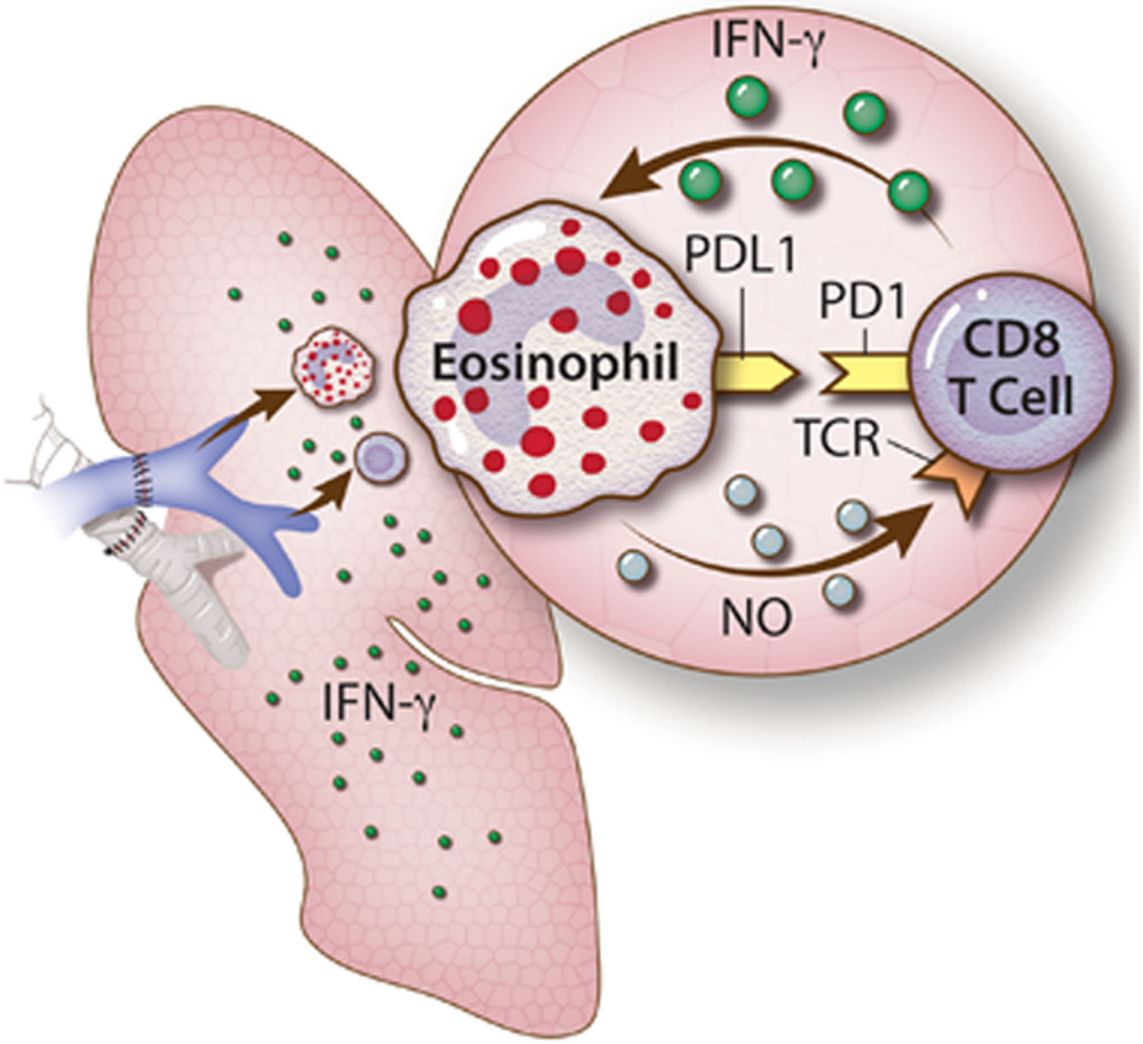

Figure 2. Mechanism of eosinophil-CD8+ T cell tolerogenic feedback loops in the lung allograft.
Effector CD8+ T cells are the major culprits in allogeneic lung graft rejection. Naïve CD8+ T cells become activated on experiencing alloantigen. This process is associated with TCR engagement and upregulation of differentiation associated molecules, such as PD-1, and the release of Th1 associated cytokines (IFN-γ and TNF-α). The Th1 cytokines released in the microenvironment milieu causes the polarization of eosinophils and their upregulation of PD-L1 and iNOS. PD-L1 binds to PD-1 to provide an immunologic synapse between eosinophil and CD8+ T cells while iNOS catalyzes the synthesis of nitric oxide (NO) that inhibits TCR signaling in a feedback loop (reproduced from data described in[74,75,82]).