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Evidence for a pro-apoptotic function of CD137 in granulocytes

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Summary

Granulocyte apoptosis is crucial for controlling granulocyte number under normal and inflammatory conditions. Reduced apoptosis of different types of granulocytes is one important mechanism for cell accumulation. Which granulocyte subtype expands is largely dependent on the cytokine milieu present at the inflammatory site. Over expression of G-CSF and GM-CSF is associated with neutrophilia, whereas over expression of IL-5 is linked to eosinophilia. Cytokine withdrawal leads to the induction of granulocyte apoptosis, a mechanism which occurs during resolution of inflammation. Besides survival factors, granulocyte apoptosis is also regulated by death factors, which belong to the tumor necrosis factor (TNF)/nerve growth factor (NGF) superfamily. Recent observations suggest that granulocytes can be activated via CD137, a member of the TNF/NGF receptor superfamily. This review summarizes our current knowledge on the potential role of CD137 in the regulation of both neutrophil and eosinophil apoptosis.

Keywords: allergy; apoptosis; CD137; cytokines; eosinophils; inflammation; neutrophils

Introduction

Receptors of the TNF/NGF family have been shown to mediate a number of physiological functions, including induction of apoptosis [1]. A subset of this family has, in addition to the cysteine rich motifs found in the extracellular domain of all members, a common intracellular sequence able to induce apoptosis in some systems and termed death domain. However, the TNF/NGF receptor family contains also non-death receptors, which lack a death domain, and therefore they may not directly activate the caspase cascade [2]. CD137, originally named "induced by lymphocyte activation" (ILA) in humans and 4-1BB in mice, represents one of the non-death receptors of the TNF/NGF receptor superfamily [3].

Whereas several transformed cells demonstrate constitutive CD137 expression, it appears that the expression in T cells, B cells, and monocytes is activation-dependent [4]. The function of CD137 is currently not completely understood. CD137 ligand or agonistic anti-CD137 mAb have been shown to enhance T cell activation *in vitro* [5] and *in vivo* [6]. In contrast, treatment of mice with anti-CD137 mAb has also been reported to abolish humoral immune responses by the induction of T helper cell anergy [7]. Moreover, CD137 may not only act as a receptor, but also as ligand. For instance, CD137 induced apoptosis in T cells [5] and activation of monocytes [8], suggesting that CD137 ligand may transduce signals into the cell initiated by CD137 protein binding. Such a mechanism is also called reverse signaling.

We have recently observed that both neutrophils [9] and eosinophils [10] can express CD137 on their surface. Interestingly, the CD137-induced intracellular pathway somehow blocked anti-apoptosis mediated by the common β-subunit of IL-3/IL-5/GM-CSF receptors in both granulocyte types in vitro. Here, we speculate that the absence of this potential anti-inflammatory mechanism might be associated with massive granulocyte accumulation and consequent tissue damage.

Overproduction of survival factors at the inflammatory site delays granulocyte apoptosis

Neutrophils and eosinophils are terminally differentiated granulocytes that are essential components of the natural immune system. Whereas neutrophils are important within the defense against bacteria and fungi, it is believed that eosinophils are required for a successful removal of at least certain parasites. On the other hand, eosinophils represent inflammatory effector cells in many allergic diseases. Apoptosis is the most common form of physiologic cell death of both neutrophils and eosinophils [11]. The phagocytic removal of apoptotic granulocytes prevents them from releasing their cytotoxic intracellular content into the extracellular space; that would occur if the cells died by necrosis or another form of cell death associated with the disruption of plasma membranes.

Both neutrophils and eosinophils have a short half-life in the absence of inflammation. Their half-life can increase under pathologic conditions, particularly at the site of inflammation. For instance, prolonged neutrophil survival *ex vivo* has been associated with many neutrophilic inflammatory responses [12]. The suppression of neutrophil apoptosis is at least partially due to the over expression of survival factors such as GM-CSF and G-CSF [12]. Similarly, delayed eosinophil apoptosis has been demonstrated in nasal polyp tissue explants *ex vivo*, and this correlated with the increased expression of IL-5 [13]. Thus it appears that quantitative and qualitative properties of the cytokine microenvironment determine how many and which effector cells accumulate in inflammatory tissues. Moreover, down regulation of survival factor expression is associated with the induction of granulocyte apoptosis [12, 13], which is important for the resolution of inflammatory responses [14]. On the other hand, there might be the possibility of granulocyte apoptosis induction even in the presence of increased levels of survival factors. For instance, TRAIL stimulation allows normal apoptosis in neutrophils in spite of permanent GM-CSF or G-CSF exposure [15]. We, therefore, searched for other mechanisms able to counter-regulate cytokine-mediated anti-apoptosis in granulocytes.

CD137 expression and function in neutrophils

It was originally reported that CD137 expression is limited to oncogenically or virally transformed or activated primary lymphocytes. We have recently published that CD137 is constitutively expressed by neutrophils [9]. This was the first report that demonstrated CD137 expression on non-activated primary cells. In terms of function, we observed that when neutrophils were simultaneously stimulated with anti-CD137 monoclonal antibody and GM-CSF, CD137 stimulation completely abrogated GM-CSF – mediated neutrophil survival and apoptosis *in vitro*. CD137 stimulation alone had no effect on the life-span of neutrophils *in vitro*. G-CSF and IFN-γ – mediated survival was not influenced by CD137 activation. This suggests that the potential CD137-mediated inhibitory effect on cytokine signal transduction is limited and does not affect all known survival factors for neutrophils.

We also observed that stimulation of neutrophils with TNF-α rapidly reduced CD137 surface protein levels. Anti-CD137 stimulation had no effect on GM-CSF-stimulated neutrophils lacking CD137, suggesting that the cytokine signals were normally transduced. Since neutrophils play a major role in bacterial inflammatory responses, we chose cystic fibrosis as a model disease to investigate TNF-α and CD137 expression *in vivo*. TNF-α was found to be highly expressed in lungs from cystic fibrosis patients but not from control individuals. TNF-α was mainly produced by epithelial cells, but also macrophages and neutrophils themselves appeared to contribute to the overall production of this cytokine. Interestingly, neutrophils derived from the inflammatory site of these patients (bronchoalveolar lavage fluid neutrophils) did not express CD137, suggesting that TNF-α also contributes to the generation of CD137-deficient neutrophils under *in vivo* conditions. It is possible that the lack of CD137 expression by neutrophils participates in the massive accumulation of these inflammatory cells in the lung of cystic fibrosis patients (Figure 1).

Moreover, T cells undergo apoptosis via reverse signaling through CD137 ligand, at least *in*

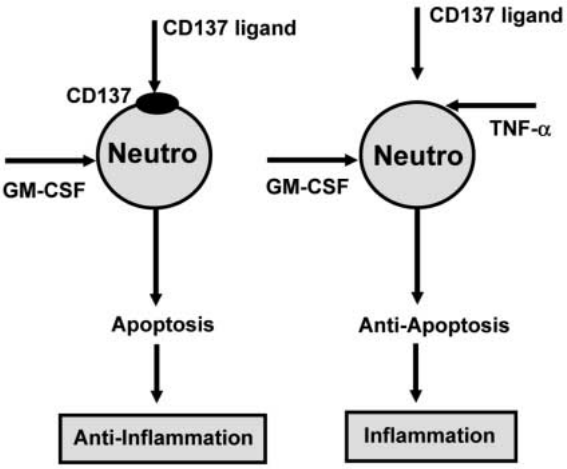

Figure 1

A proposed model showing that neutrophils undergo apoptosis in the presence of GM-CSF as long as CD137 is activated. TNF-α reduces surface CD137 expression. Therefore, GM-CSF-mediated anti-apoptosis cannot be counter-regulated by CD137 activation.

vitro [5]. Therefore, the lack of CD137 expression by neutrophils may contribute to an increased survival of cytokine-producing T cells, resulting in the maintenance of inflammation. On the other hand, it has been reported that monocytes generate increased amounts of IL-6, IL-8, and TNF-α following stimulation via CD137 ligand [8]. Therefore, the lack of CD137 expression by neutrophils in cystic fibrosis patients may also represent an anti-inflammatory mechanism.

CD137 expression and function in eosinophils

We also investigated CD137 expression and function in eosinophils. Freshly purified eosinophils from patients with atopic dermatitis and extrinsic asthma expressed significant amounts of CD137, whereas eosinophils from healthy donors and patients with non-IgE-mediated eosinophilic diseases did not express detectable levels of CD137 surface protein [10]. We also investigated whether the same difference in CD137 expression is seen in tissue eosinophils under *in vivo* conditions. As assessed by immunohistochemistry, skin eosinophils from atopic dermatitis patients and nasal polyp eosinophils from patients with extrinsic asthma expressed CD137. In contrast, tissue eosinophils from patients with intrinsic asthma did not express detectable CD137. Thus, extravasation of the eosinophils into tissues did not appear to alter CD137 expression.

As with the neutrophils, we also searched for a factor that regulates CD137 expression in eosinophils. Eosinophil differentiation and survival is regulated by IL-5, which is mostly produced by activated T helper 2 cells [16, 17]. Therefore, and not surprisingly, activated T cells have often been associated with eosinophilic allergic diseases [18]. Based on these observations we hypothesized that T cells may also secrete a cytokine, which induces CD137 expression in eosinophils. Although we observed that activated T cells release a soluble factor able to induce CD137 gene expression in eosinophils, we have not succeeded in the identification of the factor so far. Among many others we can exclude the classical T helper 2 cytokine IL-4, IL-5, and IL-13 as CD137 inducing cytokines.

We also investigated the effect of CD137 activation on the regulation of eosinophil survival and apoptosis. CD137 stimulation alone had no effect on eosinophil death and apoptosis. However, when CD137 expressing eosinophils were simultaneously stimulated with anti-CD137 monoclonal antibody and GM-CSF (or IL-5), the inhibitory effect of the survival cytokine on eosinophil apoptosis was blocked. In contrast, IFN-γ – mediated antiapoptosis was not blocked by CD137 stimulation. As in the neutrophil system, these data suggest that CD137-mediated inhibition of survival signals depends on the survival factor receptor. Whereas GM-CSF and IL-5 receptors share the common β-subunit, we know that G-CSF and IFN-γ have their own receptor molecules.

It is likely that mechanisms exist that prevent unlimited expansion of eosinophils in tissues even in the presence of high concentrations of eosinophil survival factors. Potential candidates to counter-regulate the action of survival factors are death factors. Indeed, cross-linking of Fas receptors induces apoptosis in eosinophils, both in the absence and presence of survival cytokines *in vitro* [19]. However, it is not clear whether Fas ligand bearing cells indeed kill eosinophils under *in vivo* conditions. For instance, it has been shown that nitric oxide, a mediator present in increased concentrations at allergic inflammatory sites, prevents Fas receptor-mediated death [20]. Thus, the mechanisms that limit eosinophil accumulation at inflammatory sites are only poorly understood. Our data indicate that CD137 activation may limit GM-CSF – and IL-5 – mediated antiapoptosis of eosinophils.

Today, there are not many clear-cut immunological differences known to distinguish between extrinsic and intrinsic asthma [21]. Therefore, eosinophil CD137 expression appears to be a new important marker to diagnose asthma patients. The identification of the T cell-derived CD137 inducing factor might help to understand pathogenic differences between the two subgroups of asthma in the future. Furthermore, it can be speculated that lack of CD137 expression on eosinophils in patients with intrinsic asthma contributes to the excessive tissue eosinophilia seen at least in a subgroup of the patients. The same assumption could be made for patients with idiopathic eosinophilia (Figure 2).

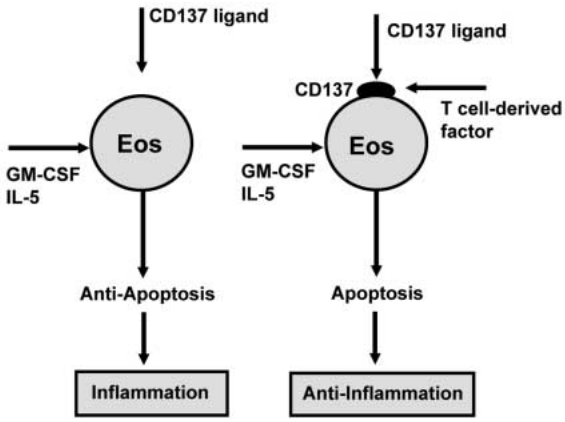

Figure 2

A proposed model showing that eosinophils demonstrate delayed apoptosis in the presence of GM-CSF and/or IL-5. A T cell-derived factor induces CD137 expression in patients with IgE-mediated allergies. Therefore, cytokine-mediated anti-apoptosis might be counter-regulated by CD137 activation to prevent unlimited expansion of eosinophils.

Conclusions

The accumulation of granulocytes during inflammation may not only have advantages for the host, since these cells also have the capacity of damaging tissues. Apoptosis of granulocytes limits their pro-inflammatory potential and is an important mechanism for the resolution of inflammatory responses. The rate of apoptosis contributes to the overall number of cells. During inflammation, granulocyte apoptosis is delayed by increased exposure to survival signals. However, a counter-regulation appears to exist and prevents unlimited accumulation of granulocytes. In vitro experiments suggest that at least one CD137-initiated signaling pathway blocks antiapoptotic effects mediated via IL-3/IL-5/GM-CSF receptors in both neutrophils and eosinophils. The absence of this potential anti-inflammatory mechanism might cause excessive accumulation of granulocytes at inflammatory sites.

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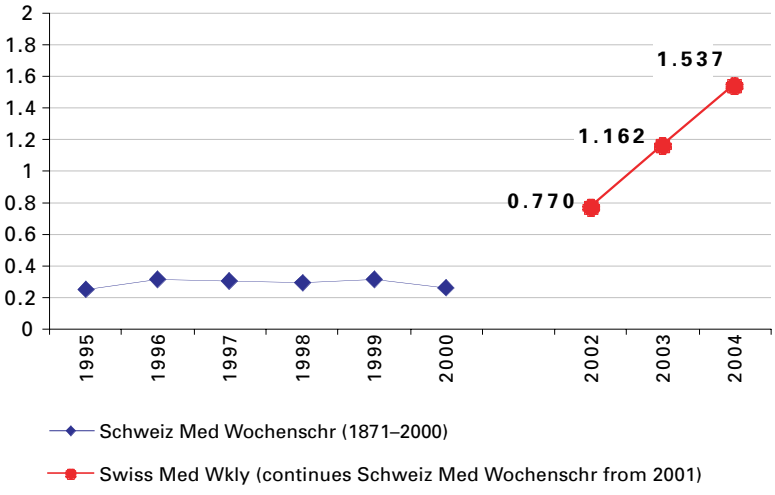

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