

Review article

Eosinophils: ‘new’ roles for ‘old’ cells

Prominent blood and tissue eosinophilia is manifested in a number of inflammatory states, particularly in allergic diseases. Eosinophils are a source of numerous cytokines and growth factors, thus in principle they can display both pro-inflammatory and anti-inflammatory activities as well as immunoregulatory ones. In this review, we will discuss the cross-talk between eosinophils and other cell types that they come in contact with in the inflammatory milieu, such as mast cells, fibroblasts and endothelial cells. ‘New’ roles for eosinophils in cancer and novel activatory signals will also be described.

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The eosinophil, which was discovered more than 120 years ago, is a ‘good looking’ blood circulating granulocyte that is associated with numerous diseases (Fig. 1) (1), and under normal conditions it is present at mucosal sites (2). Even though much is known about eosinophil morphology, differentiation, biochemistry and trafficking to the peripheral blood and tissues (2–6), there is no consensus on the so-called ‘traditional’ roles of eosinophils in the normal or diseased conditions and the eosinophil has remained quite an elusive and mysterious cell (7–13). This in spite of the number of published papers on eosinophils that have increased from approximately 600 in 1990 to nearly 1000 in 2002 (Fig. 2). Thus, it is crucial that we expand upon our current knowledge.

In this review, we will present some fresh evidence on ‘new’ roles for eosinophils in allergy, fibrosis, angiogenesis and tumors.

Eosinophils perpetuate allergic inflammation via mast cell activation and survival

In the early 1980s, it was shown that rat peritoneal mast cells can release histamine following incubation with eosinophil mediators such as major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO) but not eosinophil derived neurotoxin (EDN) (14, 15). Investigations on the possible influence of eosinophils on mast cells (Fig. 3) in the late-chronic

stages of allergic inflammation found that antigen-challenged rat peritoneal mast cells desensitized to a similar stimulus, still released histamine following incubation with MBP (16, 17). However, despite these observations on rat peritoneal mast cells, human mast cells isolated from lung and skin do not release histamine in response to MBP (18) while human heart mast cells do (19, 20).

We have recently shown that human lung-derived mast cells become responsive to MBP when cocultured with fibroblasts that sensitize them to be ‘responsive’ through the membrane form of stem cell factor (SCF) (21). SCF is a hematopoietic cytokine that is mainly responsible for mast cell differentiation, survival, proliferation, maturation, chemotaxis, and adhesion (22, 23). It also enhances IgE-dependent mediator release from various types of human mast cells (24–26).

Moreover, we demonstrated that eosinophils synthesize, store, and release SCF (27). In addition, eosinophils are a source for preformed nerve growth factor (NGF) (28) that is also a mast cell survival and activating factor (28, 29). Interestingly, NGF can act on eosinophils in an autocrine manner by activating them to release EPO (30), and the latter can activate mast cells to release histamine (18, 19), suggesting a role for eosinophil-derived NGF in mast cell–eosinophil cross talk.

These evidences all strongly suggest that eosinophils contribute, by their direct effects on mast cells, to the perpetuation of allergic inflammation.

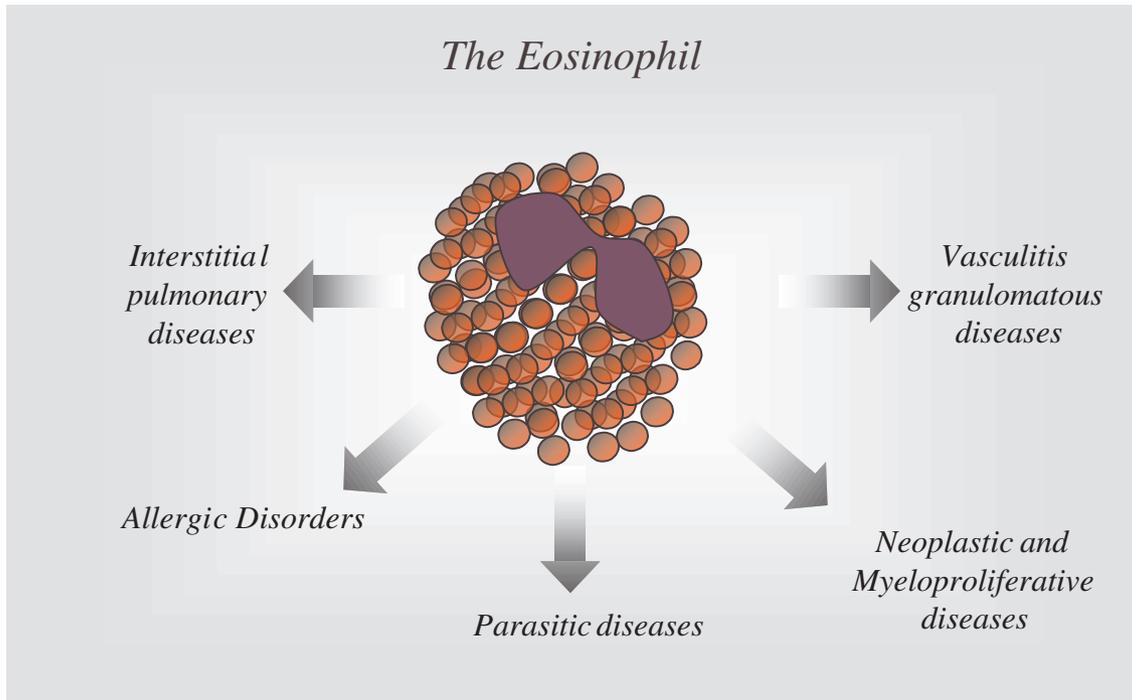


Figure 1. Some diseases in which eosinophils play roles.

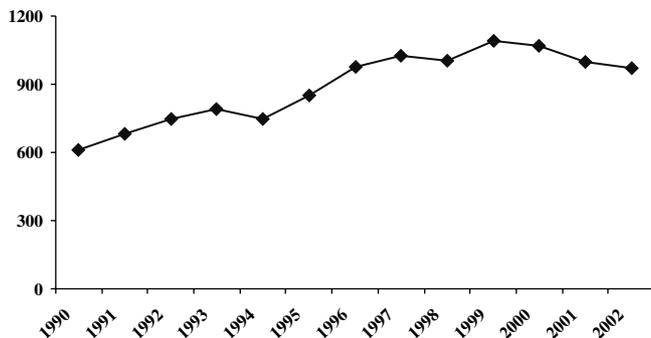


Figure 2. Number of publications (according to PubMed records) published annually on eosinophils.

Eosinophils, tissue repair, fibrosis and angiogenesis

Both eosinophils and mast cells have been associated with fibrotic conditions with different etiopathologies and more recently with the one present in asthma resulting from allergic inflammation (Fig. 4) (31). The key target and effector cells in tissue remodeling and fibrosis are the fibroblasts. They are able to migrate towards the injured area, proliferate, produce extracellular matrix, differentiate into myofibroblasts, and finally to contract the wound (31). Eosinophils can affect fibroblast properties, and hence modulate the process of tissue remodeling through the release of their distinct granule basic proteins and of an array of cytokines (30) (Fig. 3). ECP inhibits proteoglycan

degradation and increases intracellular accumulation of glycosaminoglycans in human lung fibroblasts (32). MBP and EDN display pro-fibrogenic features; the former acts synergistically with IL-1 and TGF- β to increase IL-6 production, while the latter stimulates fibroblast proliferation (33). Furthermore, it is well known that eosinophils store and release the most potent fibrogenic factor, i.e. TGF- β , and that when added to fibroblasts, eosinophils stimulate fibroblast proliferation, collagen synthesis, and lattice contraction mostly by TGF- β (34).

Moreover, eosinophils can modulate fibroblast properties by other growth factors such as fibroblast growth factor-2 (FGF-2) (35), NGF (36) and vascular endothelial growth factor (VEGF) (37). Substantial evidence shows that IL-4 and IL-13, also expressed by eosinophils, promote fibroblast functions by upregulating fibroblast chemokine and matrix protein expression (38, 39).

In human atopic skin it has been proposed that following allergen-induced IgE-dependent mast cell degranulation, infiltration of eosinophils would lead to myofibroblast formation. In such an *in vivo* system eosinophil infiltration is followed by increased expression of the matrix proteins tenascin and procollagen I (40). Eosinophils also contain preformed matrix metalloproteinases such as MMP-9, and the inhibitors of MMPs, i.e. TIMP-1 and -2 (41), indicating that they can also modulate extracellular matrix formation by their enzymes.

We have recently hypothesized that eosinophils, which contain and release VEGF (37), could also contribute to angiogenesis (Fig. 3). Eosinophils have been identified to

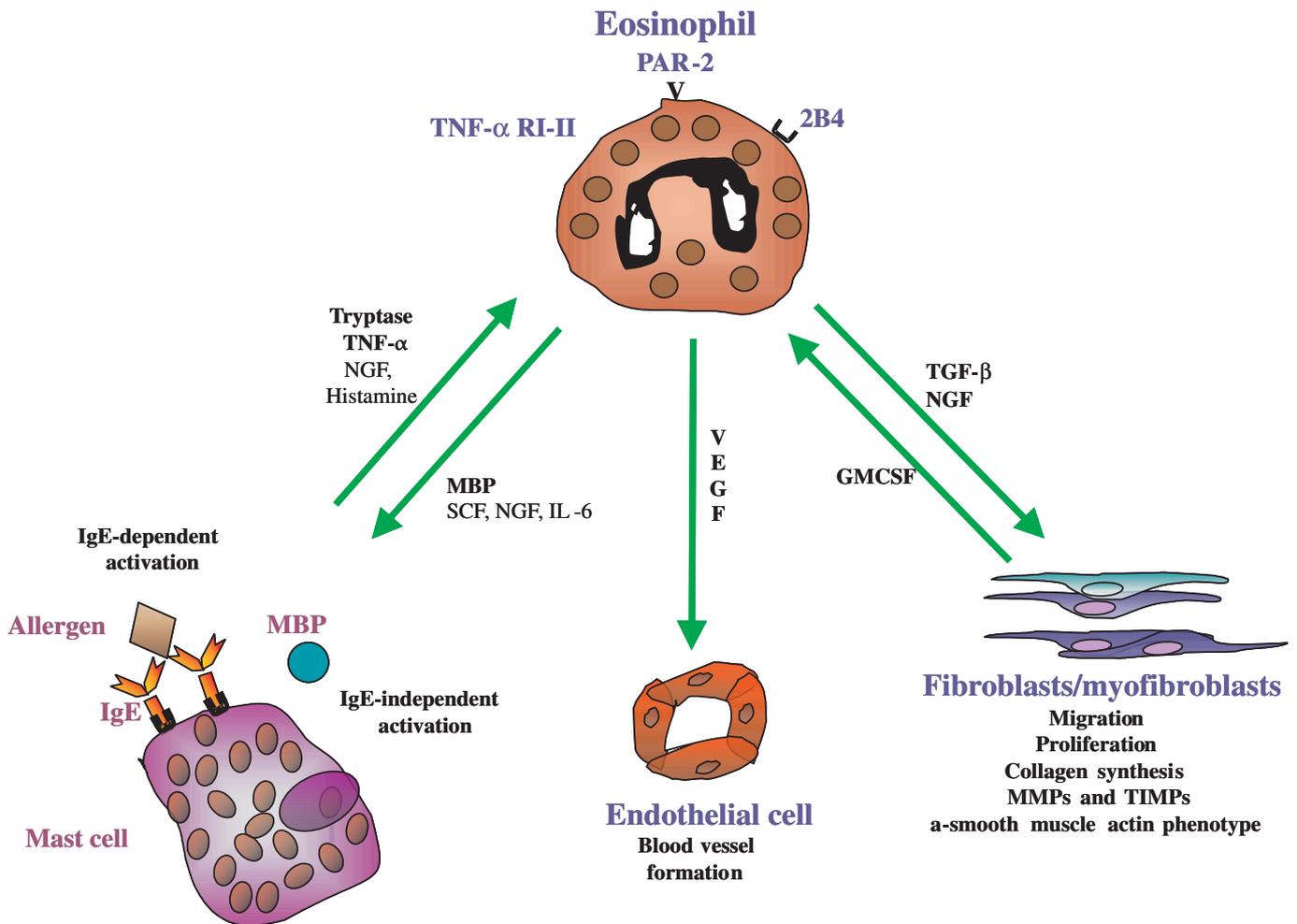


Figure 3. Eosinophils entertain a cross-talk with mast cells, fibroblasts, and endothelial cells to influence various physiological or pathological processes. *This schematic diagram represents most of the current topics investigated in our laboratory.

be positively stained for angiogenic factors such as basic-fibroblast growth factor (b-FGF) and VEGF in the submucosa of asthmatic subjects (42). In addition, platelet-derived growth factor (PDGF) and b-FGF are stored within eosinophils (43, 44). Furthermore, eosinophils synthesize and release many other pro-angiogenic cytokines such as IL-8, IL-6, TGF- β , and GM-CSF (2, 5, 45). Angiogenesis, besides having a central role in cancer and embryogenesis, is an important process both in inflammation and tissue repair/fibrosis therefore it is a topic of interest for our research.

To investigate the direct effect of eosinophil in angiogenesis we tested the effects of eosinophil sonicates on rat aorta rings embedded in collagen gel and on rat aortic endothelial cells. Our results show that eosinophil sonicates significantly enhanced rat aorta sprouting and induced endothelial cell proliferation in a concentration-dependent manner (46).

As extracellular matrix degradation is a key step in angiogenesis, we have further investigated whether

eosinophils, in addition to MMP-9, could produce also heparanase, an extracellular matrix-degrading enzyme produced by many immune and inflammatory cells (47). Interestingly, eosinophils were found to express heparanase both at the mRNA and protein levels; however, they failed to exhibit the enzymatic activity primarily because of potent heparanase-inhibiting effect of eosinophil-derived MBP (48).

This newly described information leads us to propose that eosinophils may act also as 'reparative' cells rather than only 'damage' causing ones. This concept needs further investigations in order to understand what could cause the transition from 'repair' to 'damage' and vice versa.

Eosinophils as immune effector cells towards tumors

Eosinophils have been documented to be elevated in peripheral blood and/or to infiltrate the tissue in some malignant disorders (Table 1) (49). Both aspects of

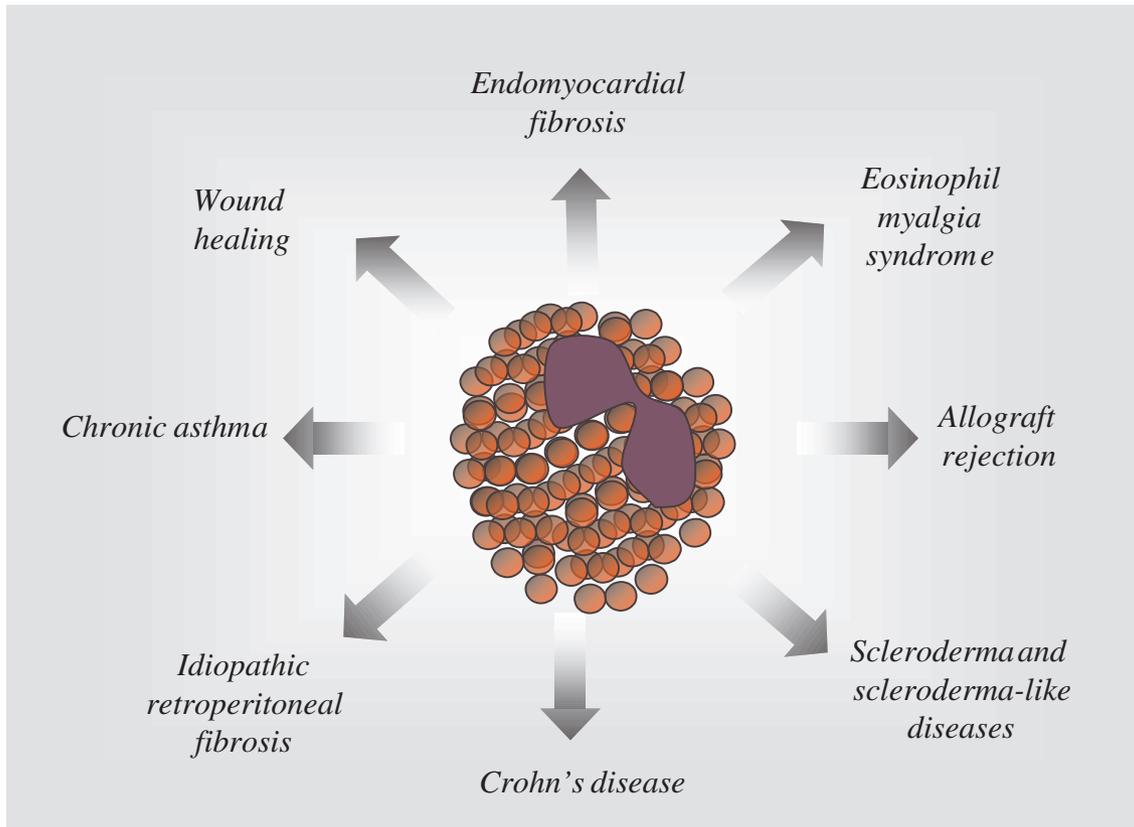


Figure 4. Some fibrotic diseases associated with eosinophils.

Table 1. Human tumors that have been associated with the presence of eosinophilia (blood and/or tissue)

Hematologic tumors	Carcinomas
Acute myelogenous leukemia	Colon
Chronic myelogenous leukemia	Stomach
Acute lymphoblastic leukemia	Pancreas
Acute myelomonocytic leukemia	Uterine cervix
Hodgkin's disease	Lung
B-cell lymphomas	Breast
T-cell lymphomas	Ovary
Plasma cell myelomas	Endothelium
AIDS-related B-cell lymphomas	Head and neck squamous carcinomas
Nodal angiolymphoid hyperplasia	Thyroid

eosinophilia, i.e. prognostic value and possible biological role in host-tumor interactions, are discussed mostly in clinical settings. Several studies have convincingly shown that tissue or blood eosinophilia is correlated with significantly better prognosis. Other studies state that presence or absence of eosinophils within tumor tissue or blood generally does not appear to have major prognostic value (49–51).

To date, the vast majority of studies in cancer immunology have focused on T helper type 1 effector mechanisms specifically on cytotoxic T cells and natural

killer cells. It is intriguing to suppose that other cells, among them eosinophils, might be involved in direct cytotoxicity toward tumors. It is well known that eosinophil granule proteins that are released upon activation are highly tumor-cytotoxic at least *in vitro* (5). Although the eosinophil role in tumor-cytotoxicity has not been entirely investigated, eosinophils have been characterized as more potent in the context of tumor cell-cytotoxicity than their fellow neutrophils (52). Eosinophil-derived EPO can synergize with macrophage reactive oxygen species to kill tumor cells (53) or catalyze the oxidation of nitrite to generate additional cytotoxic radicals (54). Thus, eosinophils might affect tumors via direct and/or indirect mechanisms. A recent work supporting these effects of eosinophils show that eosinophils might not just be bystanders due to an inflammatory process that usually accompanies the tumor, but act synergistically with macrophages against tumors. This, for example, has been described in the B16 mouse melanoma model (55). Interestingly, eradication of melanoma metastases by CD4⁺ helper type 2 cells is associated with a large influx of eosinophils into the tumor, giving yet another hint for eosinophil tumor-cytotoxicity (56). Although incubation of eosinophils with B16 melanoma cells revealed no lysis of tumor cells, eosinophil lysates are cytotoxic (56). Furthermore,

immunohistochemical staining detected eosinophil-derived MBP in lung metastatic sections. Thus, it appears that the tumor microenvironment might provide additional signals for eosinophil degranulation and tumor destruction.

Novel inhibitory/activatory receptors expressed on eosinophils: a hint for new functions?

A complex network of activating and inhibitory signals is likely to regulate the immunological or inflammatory responses coordinated by eosinophils (57). To date, several cell-surface receptor families have been described including the killer cell Ig-like receptors (KIRs), leukocyte Ig-like receptors (LIRs, also known as Ig-like transcripts, ILTs), or monocyte/macrophage Ig-like receptors (MIRs) (58). Additional receptors expressed on the myeloid cell lineage rather than the lymphoid one, such as FDF03 and the TREM family, have been recently characterized (59).

Recently, eosinophils were found to express several potential inhibitory receptors (Fig. 5). Flow cytometric analysis of human peripheral blood eosinophils revealed that all the examined populations expressed LIR-3/ILT-5 whereas only one-third of them expressed LIR-1/ILT-2 or LIR-2/ILT-4 (60). In addition to these inhibitory receptors, an activating receptor was detected (LIR-7/ILT-1) and caused eosinophil cytokine and LTC₄ release (60). Furthermore, sialic acid binding Ig-like lectins (siglecs)

were recently characterized on human eosinophils (61–63). Thus, we could speculate that there might yet be additional uncharacterized myeloid specific cell-surface inhibitory receptors.

Lately, we described that eosinophils express 2B4 (64). 2B4 belongs to the large CD2 subfamily of the Ig-superfamily. On natural killer cells, 2B4 has been described to signal either through slam-associated protein (SAP) displaying activatory features or in the absence of SAP, through SHP-1 or SHP-2 displaying inhibitory characteristics (65–67). We established that 2B4 is active on eosinophils and triggers degranulation and cytokine release. While our understanding of 2B4’s function on human eosinophils is still incomplete, we could assume that in certain conditions 2B4 signaling will exert inhibitory functions.

Finally, a recent study was performed to assess the role of SHP-1 inhibitory signaling in Th1/Th2 cell differentiation and in the development of Th2-dependent allergic airway inflammation. By using natural SHP-1 mutant mice (*me/+* mice), it has been proposed that SHP-1 controls the development of OVA-induced allergic airway inflammation (68). In fact, airway hyper-responsiveness, peribronchial and perivascular inflammation, and eosinophil infiltration was enhanced in bronchoalveolar lavage fluid of *me/+* mice when compared with wild type mice (68).

Though not addressed yet, it is intriguing to speculate the involvement of eosinophil negative signaling as a regulator of the allergic response.

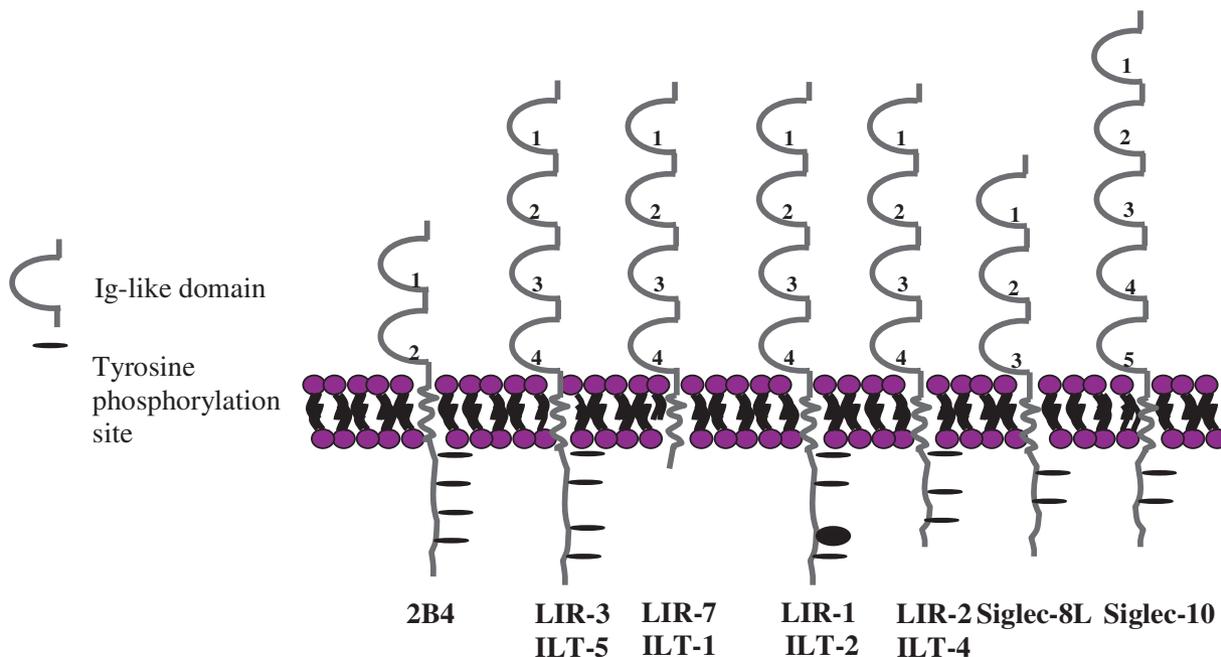


Figure 5. Receptors belonging to the Ig-superfamily recently detected on eosinophils. Several of them might display potential inhibitory functions (LIR-3/ILT-5, LIR-1/ILT-2, LIR-2/ILT-4, siglec-8, -10) whereas others (2B4, LIR-7/ILT-1) might display activating functions.

Overall, this field of negative signaling via inhibitory receptors might provide a novel therapeutic approach in eosinophil-mediated pathologies.

Conclusions

We have shown some new evidence regarding the two-faced eosinophil. On the one hand, eosinophils possess the capacity to sustain or maintain several pathologic conditions such as allergic-inflammation and fibrosis. On

the other hand, eosinophils can contribute to several physiologic or 'defensive' processes such as wound healing and tumor-cytotoxicity. Eosinophil actions are most likely to be regulated by a complex network determined by surface molecules, extracellular components, as well as additional cell-cell interactions. Since eosinophils display a wide range of functions, improved methods to evaluate their contribution in health and disease are required. Hopefully, a better understanding of this cell function in physiology and pathophysiology will lead to new clinical strategies.

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