

Current reviews of allergy and clinical immunology

(Supported by a grant from GlaxoSmithKline, Research Triangle Park, NC)

Series editor: Harold S. Nelson, MD

Verdict in the case of therapies versus eosinophils: The jury is still out

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Eosinophils are regarded by many as fundamental to the pathophysiology of allergic diseases, including asthma. An improved understanding of the mechanisms involved in these responses is therefore of great relevance to asthma pathogenesis and the development of new therapeutics. Most therapies that are effective in reducing allergic inflammation in animals and human subjects diminish tissue eosinophilia and levels of eosinophil-derived mediators. However, recent efforts with more selective eosinophil-suppressive therapies have failed to affect disease, bringing into question the role of eosinophils in asthma. This article will provide a brief overview of the role of eosinophils in allergic diseases, followed by a discussion of both eosinophil-specific and eosinophil-selective therapeutic targets, with a focus on cell-surface molecules. The known and theoretic benefits and risks of these strategies will also be covered. (*J Allergy Clin Immunol* 2004;113:3-9.)

Key words: *Eosinophil, phenotype, therapy, targets, asthma*

In October 1939, Winston Churchill, during a radio broadcast focusing on problems understanding relations with Russia, described the situation as follows: "It is a riddle wrapped in a mystery inside an enigma." He just as easily could have been talking about the biology of the eosinophil, both then and now. This cell, one of 3 terminally differentiated granulocytes, has long been recognized by its breathtaking tinctorial properties and pattern of cytoplasmic granules and lipid bodies.^{1,2} However, attempts to define the biology of this cell and its role in health and disease are still far from complete because

Abbreviations used

BAL: Bronchoalveolar lavage
CC10: Clara cell secretory 10-kd protein
ITIM: Immunoreceptor tyrosine-based inhibitory motif
LIR: Leukocyte immunoglobulin-like receptor
LT: Leukotriene
MBP: Major basic protein
Siglec: Sialic acid-binding Ig-like lectin
VLA: Very late activation antigen

efforts to target this leukocyte to fulfill the cytopathologic equivalent of Koch's postulates remain inconclusive.³⁻⁵ The purpose of this review is to briefly discuss the purported role of eosinophils in asthma and other allergic diseases. Unique and shared eosinophil-associated surface structures and other molecules that could serve as therapeutic targets will then be discussed. Knowledge gained by recent and ongoing studies with eosinophil-specific and eosinophil-selective drugs will be assessed. This will be followed by a discussion of therapeutic approaches that would be expected to have indirect, rather than direct, effects on eosinophils. Finally, the safety of chronic eosinophil suppression will be considered.

ROLE OF EOSINOPHILS IN ASTHMA AND ALLERGIC INFLAMMATION

Eosinophils contain and release a panoply of toxic pro-inflammatory mediators, including granule proteins (eg, major basic protein [MBP] and eosinophil cationic protein), reactive oxygen species, lipid mediators, and cytokines that could contribute to airways inflammation, potentially damaging airway tissues and associated nerves.^{6,7} Unlike for granule proteins, however, the production of some mediators by eosinophils (eg, leukotriene [LT] C₄, IL-4, IL-5, and eotaxin) pales in comparison with amounts produced by other cells.⁸ Another eosinophil conundrum is that the pathways triggering or regulating eosinophil mediator release in vivo remain unclear. Thus, in addition to eosinophils, other cells, including T cells, mast cells, and basophils, are believed to contribute to airway epithelial injury, smooth muscle constriction, airway hyperreactivity, excess mucus secretion, and vascular leak through more impressive production of many of these same mediators.⁹

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Disclosure of potential conflict of interest: B. S. Bochner has consultant arrangements with Aventis and Genentech/Novartis and is on the Speakers' Bureau of Merck, Aventis, and Genentech/Novartis. Under a licensing agreement between GlaxoSmithKline and The Johns Hopkins University, he is entitled to a share of royalty received by the University on the potential sales of Siglec-8 related products described in this article. He is a paid consultant to GlaxoSmithKline. The terms of this arrangement are being managed by The Johns Hopkins University in accordance with its conflict of interest policies.

Received for publication September 19, 2003; revised September 23, 2003; accepted for publication September 26, 2003. Online publication December 1, 2003.

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doi:10.1016/j.jaci.2003.09.046

The presence of tissue eosinophilia has long been used as a barometer of T_H2 -type inflammation. Numerous examples of animal models, including mice and guinea pigs, show profound allergen-induced lung eosinophilia in conjunction with worsening mucus production, airways resistance, and T_H2 cytokine generation.¹⁰ Bronchospasm and bronchial hyperreactivity can be induced in vitro and in vivo with MBP,^{11,12} and antibodies to MBP block the development of allergen-induced asthma-like changes in guinea pigs.¹³ Most allergen-provocation models correlate the presence of airway eosinophils with markers of disease,¹⁴ although there are clear examples in which lung eosinophilia can be dissociated from other markers of the asthma-like phenotype.¹⁵

Most of the evidence for a role of eosinophils in human asthma seems convincing, albeit circumstantial. Studies in human subjects during spontaneous disease exacerbations, as well as those using experimental allergen challenge of the airways, have consistently demonstrated increased levels of eosinophils in the airway lumen and parenchyma, with cell numbers correlating to disease severity.^{16,17} Deposition of MBP in asthmatic airway biopsy specimens and the ability of MBP and eosinophil peroxidase to damage airways cells in vitro implicates eosinophil degranulation products in asthma pathogenesis.^{18,19} When asthma symptoms resolve, with or without treatment, these cells and their mediators decline.²⁰ Therapies considered to be the most effective for asthma, such as glucocorticoids, have potent antieosinophil effects.²¹ Indeed, a therapeutic approach based on suppressing numbers of sputum eosinophils in the airways was shown to be more effective than those that only used standard asthma guidelines.²² Although these studies point to a pathophysiologic role for the eosinophil in asthma, it remains absolutely critical to test the "eosinophil hypothesis" more directly by attempting to generate eosinophil-focused therapies.

EOSINOPHIL SURFACE PHENOTYPE

As a result of many efforts, including international leukocyte typing workshops that assemble large panels of mAbs,²³ gene chip array studies,²⁴ and others, our knowledge of eosinophil surface and gene expression has markedly expanded over the last decade. These approaches have revealed an extensive array of surface structures, many of which had been thought to be exclusively expressed by another cell type (Fig 1).²⁵ Eosinophils express numerous adhesion molecules; receptors for cytokines, chemoattractants, and Igs; Ig superfamily members; and other transmembrane structures, including those with signal transduction capabilities. Examples of recently identified surface structures on eosinophils (some of which will be discussed in greater detail below) include the costimulatory molecules CD28 and CD86,²⁶ the proapoptotic structure sialic acid-binding Ig-like lectin 8 (Siglec-8),^{27,28} the prostaglandin D_2 receptor CRTH2,^{29,30} and several members of the inhibitory and activating leukocyte immunoglobulin-like

(LIR) receptor family (LIR1, LIR2, LIR3, and LIR7).³¹ Despite these efforts, there is still no one single marker that is uniquely expressed on the surface of eosinophils. Overall, the eosinophil has a unique pattern of cell-surface markers that more closely resembles the basophil than the neutrophil.^{8,32,33}

EOSINOPHIL-SPECIFIC TARGETS

Recently, studies in human subjects with mepolizumab, a neutralizing humanized mAb to IL-5 from Glaxo SmithKline, have raised serious concerns about the role of eosinophils in asthma. The first published study³⁴ found that a single dose of mepolizumab resulted in an 80% to 90% reduction in the number of circulating eosinophils lasting for at least 4 months in the 10 mg/kg treatment group. This was associated with a reduction in allergen-induced sputum eosinophils. However, no significant effect on the physiology of the allergen-induced acute or late-phase response was seen. A subsequent multidose study with this same humanized antibody confirmed the reduction in blood eosinophils and also showed no effect of this treatment on circulating T-cell subsets or their ability to generate cytokines.³⁵ A similar lack of effect on airway hyperreactivity was seen when IL-12, a pro- T_H1 , IFN- γ -stimulating cytokine, was administered, although effects on circulating eosinophil numbers were more modest, and side effects were frequent.³⁶ In a subsequent rostrum article in the *Journal of Allergy and Clinical Immunology*, however, potential flaws of these studies were discussed.³⁷ For example, it was noted that the Leckie study was underpowered to make any conclusion regarding efficacy. Indeed, a re-evaluation of the data suggested that anti-IL-5 appeared to protect against allergen-induced worsening of airway hyperreactivity, although the control group did not have worsened airway hyperreactivity. Ultimately, however, this anti-IL-5 antibody, as well as a competing antibody product from Schering Plough called SCH55700, failed to affect lung function in controlled clinical trials (S. Holgate, personal communication).³⁸

More recently, however, interest in the eosinophil-asthma connection has made somewhat of a comeback.^{5,14} Studies in mice³⁹ and human subjects^{40,41} comparing levels of lung parenchymal eosinophils with those in blood, bronchoalveolar lavage (BAL) fluid, or bone marrow have made a remarkable observation. Although eosinophil numbers in blood, BAL fluid, and bone marrow were reduced by 70% to 80% or more in IL-5 knockout mice or after 3 months of anti-IL-5 therapy, levels of eosinophils in lung parenchyma or in bronchial biopsy specimens were only reduced by about 50%. Levels of eosinophil cationic protein in BAL fluids were unchanged. In fact, data from mouse models suggest that only in dual IL-5/eotaxin knockout mice is lung eosinophilia and airways hyperreactivity completely eliminated. Furthermore, it appears that human eosinophils entering the airway lumen lose their surface receptors for IL-5, yet display an enhanced level of recep-

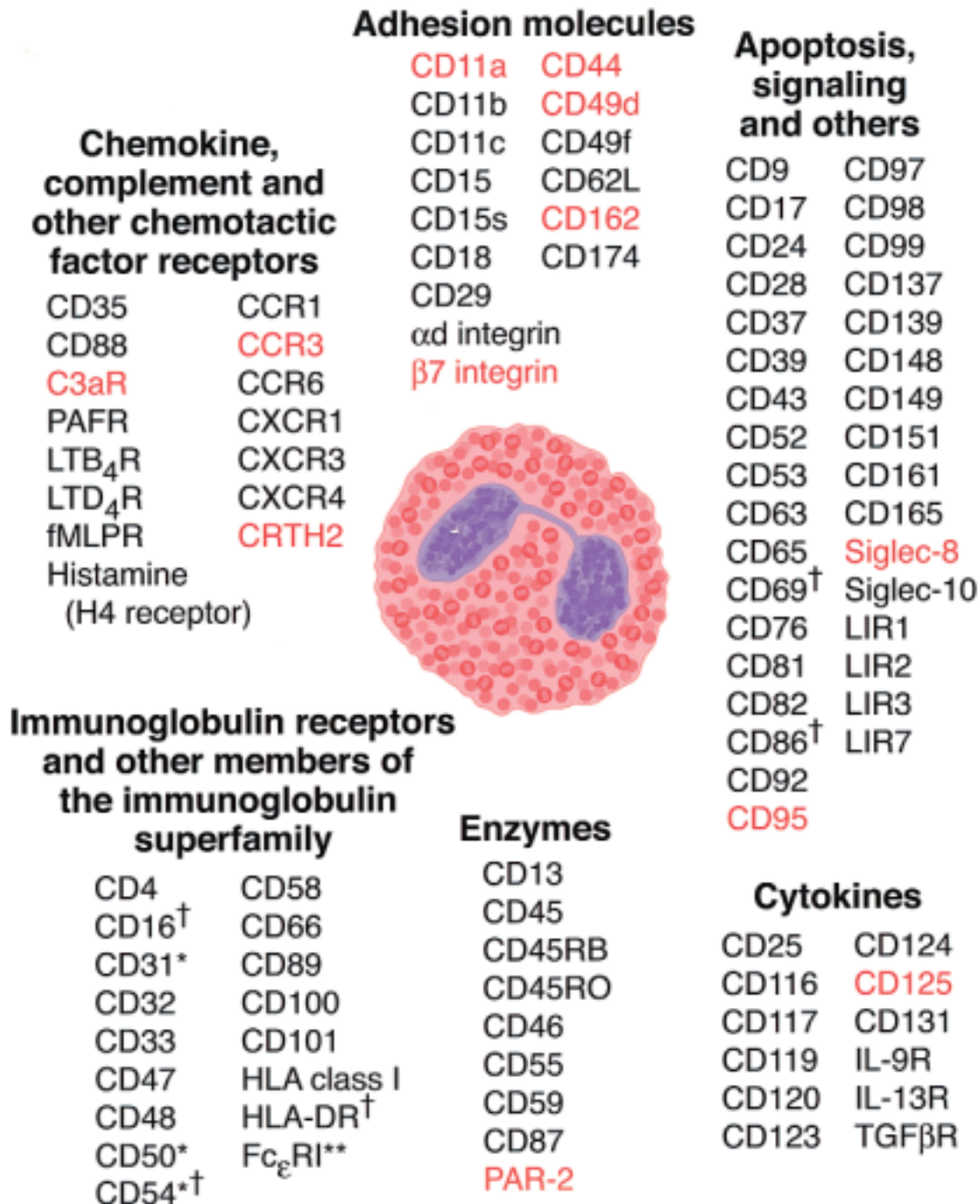


FIG 1. Examples of surface molecules expressed by human eosinophils. Although many have been identified by means of flow cytometry, the presence of some are inferred on the basis of cellular responsiveness to specific stimuli. Those molecules specifically mentioned in the text as potential eosinophil targets are shown in red. Modified with permission from Tachimoto and Bochner.²⁵ *Formerly reported to be negative; **reports (both positive and negative) are conflicting; [†]activated eosinophils; ^{††}presence based primarily or exclusively on functional data.

tors for another eosinophil-activating cytokine, GM-CSF.^{42,43} In vitro studies suggest that surface expression of IL-5 receptors on eosinophils undergo downregulation on exposure to activating cytokines, including IL-5 and GM-CSF.⁴⁴ These latest findings seem to indicate that IL-5-focused strategies might be ineffective in asthma, not

because the eosinophil is a poor target but because redundant pathways function to maintain eosinophil accumulation and activation in the lung parenchyma. Despite incomplete removal of eosinophils from the lung parenchyma, another biopsy study done after 3 months of mepolizumab found that the amount of several tissue

matix proteins, including tenascin, lumican, and procollagen III, were significantly reduced in the reticular basement membrane of the airways.⁴⁵ This was followed by studies showing IL-5 receptors on airways smooth muscle⁴⁶ and preliminary data in mice that an anti-IL-5 receptor antibody was much more effective than an anti-IL-5 antibody in allergen-driven asthma models.⁴⁷ These data implicate eosinophils, eosinophil-derived mediators, or both in airways remodeling and hint that perhaps a longer trial is needed. Whether this form of therapy will be effective for other hypereosinophilic disorders is currently being evaluated.

Because IL-5-directed therapies failed to completely inhibit eosinophils in the lungs of mice and human subjects, attempts have been made to develop other strategies. It now appears that a true “eosinophil knockout mouse” has been created.⁴⁸ This approach was based on the observation that GATA-1, a transcription factor, is required for the eosinophil lineage. By targeting a high-affinity GATA binding site in the GATA-1 promoter, investigators generated a new strain of mice with a selective and complete loss of eosinophils. Thus far, they appear otherwise healthy, and therefore these mice should be useful in defining the role of eosinophils in mouse models of parasitic, allergic, and other diseases.

EOSINOPHIL-SELECTIVE TARGETS

Molecules involved in recruitment and activation

Given the localization of eosinophils within inflamed mucosal sites, it is important to understand how these cells are preferentially recruited and localized within tissue sites. Mechanisms contributing to tissue-specific accumulation of these inflammatory cells include selective migration, increased cell survival, and decreased apoptosis.³³ Even though eosinophils share many phenotypic features with other leukocytes (Fig 1), especially basophils, including those involved in adhesion and migration, their localization and survival within a given tissue can be quite distinct.

With respect to eosinophil-selective adhesion molecules, eosinophils express very late activation antigen (VLA) 4 (CD49d/CD29) and VLA-6 (CD49f/CD29), and numerous animal studies in which antibodies or small molecules have been used to block VLA-4 have shown impressive reductions in eosinophil numbers and disease activity.⁴⁹⁻⁵² Unfortunately, efforts by Merck and Aventis in testing nebulized VLA-4 antagonists in phase II trials have not proved fruitful, perhaps in part because of inadequate dosing, short biologic half-life, or other pharmacokinetic properties. Trials with oral VLA-4 antagonists are still ongoing. Also promising is an antibody to CD49d (Antegren) from Elan Pharmaceuticals that shows efficacy in other T cell-mediated diseases, such as multiple sclerosis.⁵³ Interestingly, systemic administration of this antibody is associated with peripheral blood lymphocytosis and eosinophilia, suggesting that the therapy reduces extravasation of these cells. For eosinophilic gastrointestinal diseases, animal studies showing efficacy of

$\alpha\beta 7$ -directed therapies appear quite promising for disorders such as eosinophilic gastroenteritis.^{54,55}

Among β_2 integrins, there is much less selectivity for eosinophils. However, a recent study with a CD11a-specific antibody, efalizumab, from Genentech, showed reduced sputum eosinophilia and airway reactivity in human allergen-provocation models.⁵⁶ Targeting intercellular adhesion molecule 1, the main CD11a/CD18 ligand, also inhibits allergic inflammation in primates.⁵⁷ Among selectins, P-selectin glycoprotein ligand 1 (CD162) is relatively overexpressed on eosinophils compared with other granulocytes,⁵⁸ and in animal models P selectin-directed therapies have been shown to reduce eosinophilic inflammation.^{59,60}

It is also logical to focus on chemoattractant receptors preferentially expressed by eosinophils. Although eosinophils express a wide range of 7 transmembrane receptors, those receiving recent attention as therapeutic targets include the eotaxin family receptor CCR3, which also recognizes monocyte chemoattractant protein 4 and other chemokines. A monoclonal eotaxin antibody, CAT-213, from Cambridge Antibody Technologies, is in early clinical trials,⁶¹ and several small-molecule antagonists have recently been reported, some of which would be expected to enter clinical trials in the near future.⁶²⁻⁶⁴ Another receptor selectively expressed on eosinophils is CRTH2. Like CCR3, this receptor is also expressed on mast cells and basophils but is found on T_H2 lymphocytes more consistently than CCR3. It is a receptor for prostaglandin D₂, the major prostanoid produced by mast cells during allergic reactions.^{29,30,65} Also, like CCR3, several small-molecule antagonists have been developed and are awaiting clinical testing.⁶⁶ Other molecules, such as heparin and related proteoglycans, are capable of neutralizing a range of chemokines and other chemoattractants.⁶⁷ Finally, other receptors involved in cell trafficking with the potential to be useful eosinophil-directed targets that are preferentially, but not exclusively, expressed by eosinophils include C3a and C5a receptors (CD88),⁶⁸ LTB₄ and LTD₄ receptors,⁸ CD44,^{69,70} and the histamine H4 receptor.⁷¹

Molecules involved in apoptosis

As a result of efforts to identify novel surface molecules expressed on eosinophils, Siglec-8, formerly called sialoadhesin family member 2, was discovered.^{27,72} Siglecs are a recently designated family of cell-surface molecules that are a subset of the Ig superfamily.⁷³ Siglecs differ from traditional Ig superfamily members in several ways. Although their extracellular domains contain a variable number of Ig domains, they possess an N-terminal V-set Ig domain that binds sialic acid. In addition, Siglec cytoplasmic domains typically contain multiple tyrosine residues, including some with consensus immunoreceptor tyrosine-based inhibitory motifs (ITIMs).

By using mAbs, Siglec-8 was found to be selectively expressed on the surface of eosinophils, basophils, and mast cells.²⁷ Siglec-8 exists in 2 splice variants, termed Siglec-8 and Siglec-8L, the latter containing an identical extracellular domain but a longer cytoplasmic tail pos-

sessing 2 tyrosine-based motifs.^{74,75} The membrane-proximal motif conforms to a consensus ITIM, whereas the membrane distal motif resembles a sequence found in signaling lymphocyte activation molecule (CD150) that binds to signaling lymphocyte activation molecule-associated protein, a molecule that is abnormal in X-linked lymphoproliferative disease.⁷⁶ Of further interest is that most Siglecs are encoded on chromosome 19q13.⁷⁷ This region of chromosome 19 has received attention in the asthma community because several genetic studies show linkage of disease to this region.⁷⁸⁻⁸¹

Because Siglec-8L contains a consensus ITIM sequence, studies have begun to explore the consequences of Siglec-8 cross-linking. What has been uncovered is the observation that antibodies to Siglec-8 induce profound, caspase-dependent eosinophil apoptosis that is enhanced rather than inhibited by traditional eosinophil survival-promoting cytokines, such as IL-5 and GM-CSF.²⁸ These data suggest that Siglec-8 agonism could provide an approach to alter eosinophil survival.

In addition to the Siglecs is a family of LIRs which share many structural characteristics with those of the Siglecs, except that the LIRs lack the N-terminal sialic acid-binding domain. Among the LIR family, eosinophils can express LIR1, LIR2, LIR3, and LIR7, with the latter 2 being most consistently expressed.³¹ LIR7 has a cytoplasmic immunoreceptor tyrosine-based activation motif domain and has been shown to have eosinophil-activating activities. The other 3 LIRs contain cytoplasmic ITIM domains, suggesting that activation through these surface structures might downregulate eosinophil function, although this needs further study.

A host of additional, albeit less selective, pathways have been identified for induction of eosinophil apoptosis. These include the use of antibodies to Fas (CD95)^{82,83} or CD69, an eosinophil activation marker.^{69,84} The cytokine TGF- β induces eosinophil apoptosis in vitro,⁸⁵ as do drugs such as lidocaine⁸⁶ and glucocorticoids.⁸⁷ Recent studies looking at glucocorticoid-induced genes by array technologies might suggest additional ways of activating eosinophil death pathways.⁸⁸

Examples of other approaches that directly or indirectly reduce eosinophils

A mouse genetically deficient in the surfactant protein Clara cell secretory 10-kd protein (CC10) displayed exaggerated allergic eosinophilic airways inflammation, suggesting that CC10 might have anti-inflammatory effects.⁸⁹ A recombinant form of CC10 from a company called Claragen was approved under orphan drug status for the treatment of lung complications in premature infants. Other studies have also shown that anti-IgE therapy reduces circulating eosinophil numbers,⁹⁰ but this is unlikely to be due to a direct effect because eosinophils have little, if any, IgE and Fc ϵ RI on their surface.^{91,92} Mice deficient in histamine synthesis,⁹³ C3a receptors,⁹⁴ and protease-activated receptor 2⁹⁵ are additional examples of where elimination of particular molecules can have profound effects, either direct or indirect, on allergic eosinophilic inflammation.

SAFETY CONCERNS WITH CHRONIC INHIBITION OF EOSINOPHILS

Ultimately, even if eosinophil-directed therapies are shown to have beneficial effects in asthma and other allergic disorders, chronic eosinophil inhibition could lead to serious side effects. Fortunately, administration of anti-IL-5 antibody has been extremely well tolerated, but this approach did not fully suppress eosinophils in all tissues. On the basis of animal and in vitro data, the most likely detrimental effects, if any, that chronic systemic eosinophil suppression might cause would include enhanced susceptibility to certain helminthic parasites⁹⁶ and a theoretic increased risk in viral infections. The latter is a theoretical concern because of the known RNase activity of eosinophil granule proteins and their potential beneficial antiviral effects against RNA-based viruses, such as respiratory syncytial virus.⁹⁷

CONCLUSIONS

Human eosinophils display a wide range of surface structures, including those involved in adhesion, apoptosis, and signaling (Fig 1). They are also equipped with surface enzymes, receptors for cytokines, chemokines, complement proteins, and other chemoattractants, as well as a wide array of Ig receptors and other structures belonging to the Ig gene superfamily. Hopefully, future studies targeting eosinophils and eosinophil-related structures will ultimately clarify the role of this enigmatic cell in asthma and allergic inflammation. In the meantime, to paraphrase Mark Twain, reports of the death of the "eosinophil hypothesis" in asthma have been greatly exaggerated.

REFERENCES

- Weller PF. Human eosinophils. *J Allergy Clin Immunol* 1997;100:283-7.
- Rothenberg ME. Mechanisms of disease: eosinophilia. *N Engl J Med* 1998;338:1592-600.
- Menzies-Gow A, Robinson DS. Eosinophils, eosinophilic cytokines (interleukin-5), and antieosinophilic therapy in asthma. *Curr Opin Pulm Med* 2002;8:33-8.
- Kay AB, Menzies-Gow A. Eosinophils and interleukin-5: the debate continues. *Am J Respir Crit Care Med* 2003;167:1586-7.
- Foster PS, Hogan SP, Yang M, Mattes J, Young IG, Matthaei KI, et al. Interleukin-5 and eosinophils as therapeutic targets for asthma. *Trends Mol Med* 2002;8:162-7.
- Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000;105:651-63.
- Kingham PJ, Costello RW, McLean WG. Eosinophil and airway nerve interactions. *Pulm Pharmacol Ther* 2003;16:9-13.
- Kita H, Adolphson CR, Gleich GJ. Biology of eosinophils. In: Adkinson NF Jr, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER, eds. *Allergy: principles and practice*. 6th ed. Philadelphia: Mosby; 2003. p. 305-32.
- Barnes P. New directions in allergic diseases: mechanism-based anti-inflammatory therapies. *J Allergy Clin Immunol* 2000;106:5-16.
- Canning BJ. Modeling asthma and COPD in animals: a pointless exercise? *Curr Opin Pharmacol* 2003;3:244-50.
- Flavahan NA, Slifman NR, Gleich GJ, Vanhoutte PM. Human eosinophil major basic protein causes hyperactivity of respiratory smooth muscle. *Am Rev Respir Dis* 1988;138:685-8.
- Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991;87:1470-3.

13. Lefort J, Nahori MA, Ruffie C, Vargaftig BB, Pretolani M. In vivo neutralization of eosinophil-derived major basic protein inhibits antigen-induced bronchial hyperreactivity in sensitized guinea pigs. *J Clin Invest* 1996;97:1117-21.
14. Zimmermann N, Hershey GK, Foster PS, Rothenberg ME. Chemokines in asthma: cooperative interaction between chemokines and IL-13. *J Allergy Clin Immunol* 2003;111:227-43.
15. Wills-Karp M, Luyimbazi J, Xu XY, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-61.
16. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
17. Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. *Am J Respir Crit Care Med* 2000;161:64-72.
18. Filley WV, Holley KE, Kephart GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet* 1982;2:11-5.
19. Agosti JM, Altman LC, Ayars GH, Loegering DA, Gleich GJ, Klebanoff SJ. The injurious effect of eosinophil peroxidase, hydrogen peroxide, and halides on pneumocytes. *J Allergy Clin Immunol* 1987;79:496-504.
20. Pizzichini E, Pizzichini MM, Efthimiadis A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. *J Allergy Clin Immunol* 1997;99:539-44.
21. Gleich GJ, Hunt LW, Bochner BS, Schleimer RP. Glucocorticoid effects on human eosinophils. In: Schleimer RP, Busse WW, O'Byrne P, eds. *Inhaled glucocorticoids in asthma: mechanisms and clinical actions*. New York: Marcel Dekker, Inc; 1996. p. 279-308.
22. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715-21.
23. Ebisawa M, Schleimer RP, Bickel C, Bochner BS. Phenotyping of purified human peripheral blood eosinophils using the blind panel mAb. In: Schlossman S, Boumsell L, Gilks W, Harlan J, Kishimoto T, Morimoto C, et al, eds. *Leukocyte typing V: white cell differentiation antigens*. New York: Oxford University Press; 1995. p. 1036-8.
24. Temple R, Allen E, Fordham J, Phipps S, Schneider HC, Lindauer K, et al. Microarray analysis of eosinophils reveals a number of candidate survival and apoptosis genes. *Am J Respir Cell Mol Biol* 2001;25:425-33.
25. Tachimoto H, Bochner BS. The surface phenotype of human eosinophils. *Chem Immunol* 2000;76:45-62.
26. Woerly G, Roger N, Loiseau S, Dombrowicz D, Capron A, Capron M. Expression of CD28 and CD86 by human eosinophils and role in the secretion of type 1 cytokines (interleukin 2 and interferon gamma): inhibition by immunoglobulin A complexes. *J Exp Med* 1999;190:487-95.
27. Kikly KK, Bochner BS, Freeman S, Tan KB, Gallagher KT, D'Alessio K, et al. Identification of SAF-2, a novel siglec expressed on eosinophils, mast cells and basophils. *J Allergy Clin Immunol* 2000;105:1093-100.
28. Nutku E, Aizawa H, Hudson SA, Bochner BS. Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis. *Blood* 2003;101:5014-20.
29. Nagata K, Hirai H, Tanaka K, Ogawa K, Aso T, Sugamura K, et al. CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). *FEBS Lett* 1999;459:195-9.
30. Gervais FG, Cruz RPG, Chateanuneuf A, Gale S, Sawyer N, Nantel F, et al. Selective modulation of chemokinesis, degranulation, and apoptosis in eosinophils through the PGD2 receptors CRTH2 and DP. *J Allergy Clin Immunol* 2001;108:982-8.
31. Tedla N, Bandeira-Melo C, Tassinari P, Sloane DE, Samplaski M, Cosman D, et al. Activation of human eosinophils through leukocyte immunoglobulin-like receptor 7. *Proc Natl Acad Sci U S A* 2003;100:1174-9.
32. Bochner BS, Schroeder J. Basophils. In: Austen KF, Frank MM, Atkinson JP, Cantor H, eds. *Samter's immunological diseases*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 244-53.
33. Bochner BS, Schleimer RP. Mast cells, basophils, and eosinophils: distinct but overlapping pathways for recruitment. *Immunol Rev* 2001;179:5-15.
34. Leckie MJ, Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.
35. Buttner C, Lun A, Spletstoesser T, Kunkel G, Renz H. Monoclonal anti-interleukin-5 treatment suppresses eosinophil but not T-cell functions. *Eur Respir J* 2003;21:799-803.
36. Bryan SA, O'Connor BJ, Matti S, Leckie MJ, Kanabar V, Khan J, et al. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2149-53.
37. O'Byrne PM, Inman MD, Parameswaran K. The trials and tribulations of IL-5, eosinophils, and allergic asthma. *J Allergy Clin Immunol* 2001;108:503-8.
38. Kips JC, O'Connor BJ, Langley SJ, Woodcock A, Kerstjens HA, Postma DS, et al. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003;167:1655-9.
39. Mattes J, Yang M, Mahalingam S, Kuehr J, Webb DC, Simson L, et al. Intrinsic defect in T cell production of interleukin (IL)-13 in the absence of both IL-5, and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma. *J Exp Med* 2002;195:1433-44.
40. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199-204.
41. Menzies-Gow A, Flood-Page P, Sehmi R, Burman J, Hamid Q, Robinson DS, et al. Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol* 2003;111:714-9.
42. Liu LY, Sedgwick JB, Bates ME, Vrtis RF, Gern JE, Kita H, et al. Decreased expression of membrane IL-5 receptor alpha on human eosinophils: I. Loss of membrane IL-5 receptor alpha on airway eosinophils and increased soluble IL-5 receptor alpha in the airway after allergen challenge. *J Immunol* 2002;169:6452-8.
43. Liu LY, Sedgwick JB, Bates ME, Vrtis RF, Gern JE, Kita H, et al. Decreased expression of membrane IL-5 Receptor alpha on human eosinophils: II. IL-5 down-modulates its receptor via a proteinase-mediated process. *J Immunol* 2002;169:6459-66.
44. Gregory B, Kirchem A, Phipps S, Gevaert P, Pridgeon C, Rankin SM, et al. Differential regulation of human eosinophil IL-3, IL-5, and GM-CSF receptor alpha-chain expression by cytokines: IL-3, IL-5, and GM-CSF down-regulate IL-5 receptor alpha expression with loss of IL-5 responsiveness, but up-regulate IL-3 receptor alpha expression. *J Immunol* 2003;170:5359-66.
45. Phipps S, Flood-Page P, Menzies-Gow A, Wangoo A, Barnes N, Barkans J, et al. Anti-IL-5 (mepolizumab) reduces the expression of tenascin, procollagen III and lumican in the reticular basement membrane of human atopic asthmatics [abstract]. *J Allergy Clin Immunol* 2003;111(suppl):S278.
46. Rizzo CA, Yang R, Greenfeder S, Egan RW, Pauwels RA, Hey JA. The IL-5 receptor on human bronchus selectively primes for hyperresponsiveness. *J Allergy Clin Immunol* 2002;109:404-9.
47. Sugiyama K, Cheng G, Aoki Y, Fukuda T. Anti-IL-5 receptor antibody, but not anti-IL-5 antibody, inhibits airway hyperresponsiveness in murine model of chronic asthma [abstract]. *Am J Respir Crit Care Med* 2002;167:A494.
48. Yu C, Cantor AB, Yang H, Browne C, Wells RA, Fujiwara Y, et al. Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. *J Exp Med* 2002;195:1387-95.
49. Kudlacz E, Whitney C, Andresen C, Duplantier A, Beckius G, Chupak L, et al. Pulmonary eosinophilia in a murine model of allergic inflammation is attenuated by small molecule $\alpha 4\beta 1$ antagonists. *J Pharmacol Exp Ther* 2002;301:747-52.
50. Bochner BS. Leukocyte recruitment in allergic pulmonary inflammation. In: Eissa T, Huston D, eds. *Therapeutic targets of airway inflammation*. New York: Marcel Dekker, Inc; 2003. p. 223-47.
51. Koo GC, Shah K, Ding GJ, Xiao J, Wnek R, Doherty G, et al. A small molecule very late antigen-4 antagonist can inhibit ovalbumin-induced lung inflammation. *Am J Respir Crit Care Med* 2003;167:1400-9.
52. Renzetti L, Tannu S, Connaughton S, Sidduri A, Tilley J, Cavallo G. Attenuation of airway inflammation and hyperresponsiveness in murine and primate models of asthma by Ro 27-0608, a dual $\alpha 4\beta 1/\alpha 4\beta 7$ integrin antagonist [abstract]. *Am J Respir Crit Care Med* 2003;167:A640.
53. Miller DH, Khan OA, Sheremata WA, Blumhardt LD, Rice GP, Libonati MA, et al. A controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2003;348:15-23.

54. Mishra A, Hogan SP, Lee JJ, Foster PS, Rothenberg ME. Fundamental signals that regulate eosinophil homing to the gastrointestinal tract. *J Clin Invest* 1999;103:1719-27.
55. Rothenberg ME. Eosinophilic gastrointestinal disorders. *J Allergy Clin Immunol* 2004. In press.
56. Gauvreau GM, Becker AB, Boulet L-P, Chakir J, Fick RB, Greene WL, et al. The effects of an anti-CD11a mAb, efalizumab, on allergen-induced airway responses and airway inflammation in subjects with atopic asthma. *J Allergy Clin Immunol* 2003. In press.
57. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Inter-cellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990;247:456-9.
58. Davenport KL, Brummet ME, Sterbinsky SA, Mayer RJ, Bochner BS. Activation of human leukocytes reduces surface P-selectin glycoprotein ligand-1 (PSGL-1, CD162) and adhesion to P-selectin in vitro. *J Immunol* 2000;165:2764-72.
59. Strauss EC, Larson KA, Brenneise I, Foster CS, Larsen GR, Lee NA, et al. Soluble P-selectin glycoprotein ligand 1 inhibits ocular inflammation in a murine model of allergy. *Invest Ophthalmol Vis Sci* 1999;40:1336-42.
60. Broide DH, Humber D, Sullivan S, Sriramaraop P. Inhibition of eosinophil rolling and recruitment in P-selectin- and intracellular adhesion molecule-1-deficient mice. *Blood* 1998;91:2847-56.
61. Pereira S, Clark T, Darby Y, Salib R, Salagean M, Hewitt L, et al. Effects of anti-eotaxin monoclonal antibody CAT-213 on allergen-induced rhinitis [abstract]. *J Allergy Clin Immunol* 2003;111(suppl):S268.
62. White JR, Lee JM, Dede K, Imburgia CS, Jurewicz AJ, Chan G, et al. Identification of potent, selective non-peptide CC chemokine receptor-3 antagonist that inhibits eotaxin-, eotaxin-2-, and monocyte chemotactic protein-4-induced eosinophil migration. *J Biol Chem* 2000;275:36626-31.
63. Dhanak D, Christmann LT, Darcy MG, Keenan RM, Knight SD, Lee J, et al. Discovery of potent and selective phenylalanine derived CCR3 receptor antagonists. *Bioorg Med Chem Lett* 2001;11:1445-50.
64. Warrior U, McKeegan EM, Rottinghaus SM, Garcia L, Traphagen L, Grayson G, et al. Identification and characterization of novel antagonists of the CCR3 receptor. *J Biomol Screen* 2003;8:324-31.
65. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 2001;193:255-61.
66. Mitsumori S, Tsuru T, Honma T, Hiramatsu Y, Okada T, Hashizume H, et al. Synthesis and biological activity of various derivatives of a novel class of potent, selective, and orally active prostaglandin D2 receptor antagonists. 2. 6,6-Dimethylbicyclo[3.1.1]heptane derivatives. *J Med Chem* 2003;46:2446-55.
67. Culley FJ, Fadlon EJ, Kirchem A, Williams TJ, Jose PJ, Pease JE. Proteoglycans are potent modulators of the biological responses of eosinophils to chemokines. *Eur J Immunol* 2003;33:1302-10.
68. DiScipio RG, Daffern PJ, Jagels MA, Broide DH, Sriramaraop P. A comparison of C3a and C5a-mediated stable adhesion of rolling eosinophils in postcapillary venules and transendothelial migration in vitro and in vivo. *J Immunol* 1999;162:1127-36.
69. Matsumoto K, Appiah-Pippin J, Schleimer RP, Bickel CA, Beck LA, Bochner BS. CD44 and CD69 represent different types of cell surface activation markers for human eosinophils. *Am J Respir Cell Mol Biol* 1998;18:860-6.
70. Katoh S, Matsumoto N, Kawakita K, Tominaga A, Kincade PW, Matsukura S. A role for CD44 in an antigen-induced murine model of pulmonary eosinophilia. *J Clin Invest* 2003;111:1563-70.
71. O'Reilly M, Alpert R, Jenkinson S, Gladue RP, Foo S, Trim S, et al. Identification of a histamine H4 receptor on human eosinophils—role in eosinophil chemotaxis. *J Recept Signal Transduct Res* 2002;22:431-48.
72. Floyd H, Ni J, Cornish AL, Zeng Z, Liu D, Carter KC, et al. Siglec-8: a novel eosinophil-specific member of the immunoglobulin superfamily. *J Biol Chem* 2000;275:861-6.
73. Crocker PR, Varki A. Siglecs in the immune system. *Immunology* 2001;103:137-45.
74. Foussias G, Yousef GM, Diamandis EP. Molecular characterization of a Siglec-8 variant containing cytoplasmic tyrosine-based motifs, and mapping of the Siglec-8 gene. *Biochem Biophys Res Commun* 2000;278:775-81.
75. Aizawa H, Plitt J, Bochner BS. Human eosinophils express two siglec-8 splice variants. *J Allergy Clin Immunol* 2002;109:176.
76. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* 1998;395:462-9.
77. Yousef GM, Ordon MH, Foussias G, Diamandis EP. Genomic organization of the siglec gene locus on chromosome 19q13.4 and cloning of two new siglec pseudogenes. *Gene* 2002;286:259-70.
78. Asthma TCSotGo. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nat Genet* 1997;15:389-92.
79. Ober C, Cox NJ, Abney M, Di Rienzo A, Lander ES, Changvakeket B, et al. Genome-wide search for asthma susceptibility loci in a founder population. *Hum Mol Genet* 1998;7:1393-8.
80. Dizier MH, Besse-Schmittler C, Guilloud-Bataille M, Annesi-Maesano I, Boussaha M, Bousquet J, et al. Genome screen for asthma and related phenotypes in the French EGEA study. *Am J Respir Crit Care Med* 2000;162:1812-8.
81. Venanzi S, Malerba G, Galavotti R, Lauciello MC, Trabetti E, Zanoni G, et al. Linkage to atopy on chromosome 19 in north-eastern Italian families with allergic asthma. *Clin Exp Allergy* 2001;31:1220-4.
82. Matsumoto K, Schleimer RP, Saito H, Iikura Y, Bochner BS. Induction of apoptosis in human eosinophils by anti-fas antibody treatment in vitro. *Blood* 1995;86:1437-43.
83. Tsuyuki S, Bertrand C, Erard F, Trifilieff A, Tsuyuki J, Wesp M, et al. Activation of the fas receptor on lung eosinophils leads to apoptosis and the resolution of eosinophilic inflammation of the airways. *J Clin Invest* 1995;96:2924-31.
84. Walsh GM, Williamson ML, Symon FA, Willars GB, Wardlaw AJ. Ligation of CD69 induces apoptosis and cell death in human eosinophils cultured with granulocyte-macrophage colony-stimulating factor. *Blood* 1996;87:2815-21.
85. Alam R, Forsythe P, Stafford S, Fukuda Y. Transforming growth factor beta abrogates the effects of hematopoietins on eosinophils and induces their apoptosis. *J Exp Med* 1994;179:1041-5.
86. Okada S, Hagan JB, Kato M, Bankers-Fulbright JL, Hunt LW, Gleich GJ, et al. Lidocaine and its analogues inhibit IL-5-mediated survival and activation of human eosinophils. *J Immunol* 1998;160:4010-7.
87. Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. *J Allergy Clin Immunol* 1994;94:1202-13.
88. Chauhan S, Leach CH, Kunz S, Bloom JW, Miesfeld RL. Glucocorticoid regulation of human eosinophil gene expression. *J Steroid Biochem Mol Biol* 2003;84:441-52.
89. Chen LC, Zhang Z, Myers AC, Huang SK. Cutting edge: altered pulmonary eosinophilic inflammation in mice deficient for Clara cell secretory 10-kDa protein. *J Immunol* 2001;167:3025-8.
90. Noga O, Hanf G, Kunkel G. Immunological and clinical changes in allergic asthmatics following treatment with omalizumab. *Int Arch Allergy Immunol* 2003;131:46-52.
91. Kita H, Kaneko M, Bartemes KR, Weiler DA, Schimming AW, Reed CE, et al. Does IgE bind to and activate eosinophils from patients with allergy? *J Immunol* 1999;162:6901-11.
92. Seminario M-C, Saini SS, MacGlashan DW Jr, Bochner BS. Intracellular expression and release of Fc*RIα by human eosinophils. *J Immunol* 1999;162:6893-900.
93. Kozma GT, Losonczy G, Keszei M, Komlosi Z, Buzas E, Pallinger E, et al. Histamine deficiency in gene-targeted mice strongly reduces antigen-induced airway hyper-responsiveness, eosinophilia and allergen-specific IgE. *Int Immunol* 2003;15:963-73.
94. Drouin SM, Corry DB, Hollman TJ, Kildsgaard J, Wetsel RA. Absence of the complement anaphylatoxin C3a receptor suppresses Th2 effector functions in a murine model of pulmonary allergy. *J Immunol* 2002;169:5926-33.
95. Schmidlin F, Amadesi S, Dabbagh K, Lewis DE, Knott P, Bunnett NW, et al. Protease-activated receptor 2 mediates eosinophil infiltration and hyperreactivity in allergic inflammation of the airway. *J Immunol* 2002;169:5315-21.
96. Klion AD, Nutman TB. The role of eosinophils in host defense against helminth parasites. *J Allergy Clin Immunol* 2004. In press.
97. Rosenberg HF, Domachowski JB. Eosinophils, eosinophil ribonucleases, and their role in host defense against respiratory virus pathogens. *J Leukoc Biol* 2001;70:691-8.