

# The Enigmatic Charcot-Leyden Crystal Protein (Galectin-10): Speculative Role(s) in the Eosinophil Biology and Function

Christine A Clarke<sup>1,3</sup>, Clarence M Lee<sup>2</sup> and Paulette M Furbert-Harris<sup>1,3,4\*</sup>

<sup>1</sup>Department of Microbiology, Howard University College of Medicine, Washington, D.C., USA

<sup>2</sup>Department of Biology, Howard University College of Arts and Sciences, Washington, D.C., USA

<sup>3</sup>Howard University Cancer Center, Howard University College of Medicine, Washington, D.C., USA

<sup>4</sup>National Human Genome Center, Howard University College of Medicine, Washington, D.C., USA

\*Corresponding author: Furbert-Harris PM, Department of Microbiology, Howard University Cancer Center, 2041 Georgia Avenue NW, Room #530, 20060, Washington, D.C., USA, Tel: 202-806-7722, Fax: 202-667-1686; Email: [pfurbertharris@gmail.com](mailto:pfurbertharris@gmail.com)

Received date: February 22, 2015, Accepted date: April 25, 2015, Published date: April 29, 2015

Copyright: © 2015 Clarke CA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## Abstract

Eosinophilic inflammation in peripheral tissues is typically marked by the deposition of a prominent eosinophil protein, Galectin-10, better known as Charcot-Leyden crystal protein (CLC). Unlike the eosinophil's four distinct toxic cationic proteins and enzymes [major basic protein (MBP), eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO)], there is a paucity of information on the precise role of the crystal protein in the biology of the eosinophil. While its clinical significance at inflammatory foci remains highly speculative, its relative abundance (~10% of total eosinophil protein), as well as its dual nuclear and cytosolic localization is, however, suggestive of its biological and functional significance. In this article, we present a short review of the Charcot-Leyden crystal protein, specifically highlighting its most recently delineated modulatory role in regulatory T lymphocytes, and its speculative intracellular and extracellular role(s) in eosinophil function or associated inflammatory responses.

**Keywords:** Charcot-Leyden crystal protein; Eosinophil; Galectin; Regulatory T lymphocyte; Inflammation

## Introduction

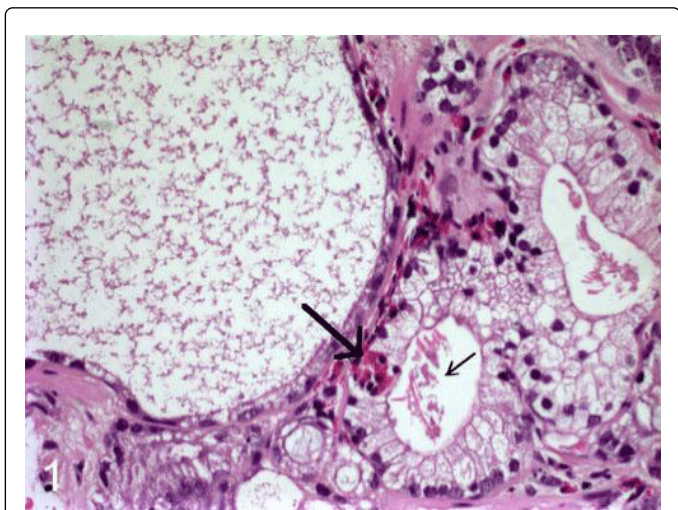
Eosinophils are bone marrow-derived granulocytes [1] that contribute to the pathogenesis of allergic, inflammatory, and immunoregulatory responses through the release of toxic proteins, preformed cytokines, chemokines, growth factors, and lipid mediators. They are activated in response to infection and tissue damage; and, in multiple disease states, inflammatory mediators stimulate their migration from the bone marrow, and their localization to the affected sites [2]. Eosinophil infiltration and localization is characterized by the presence and persistence of the enigmatic autocrystallizing Charcot-Leyden crystal protein, a member of the galectin family of carbohydrate-binding proteins that have emerged as bioactive molecules with powerful immuno-regulatory functions. The galectin family includes 15 members that are characterized by galactose-binding domains and are widely expressed in diverse cell types [3]. Different members of the glycoprotein family have been shown to positively or negatively modulate multiple steps of the inflammatory response, including cell trafficking, cell survival, pro-inflammatory cytokine secretion, cell growth and regulation [4], cell adhesion and aggregation, and tumor cell apoptosis [5]. In addition, several galectins are currently being investigated as potential therapeutic targets for immunologically-based pathologies, such as hematological malignancies [6], asthma, infections [7], autoimmune disorders and cancer [8]. Despite the explosion of information on these intriguing proteins in pathological states, particularly inflammation, fibrosis, and cancer [3], the precise role of galectin-10 in the eosinophil biology still remains to be ascertained. Its selective localization in several host

immune cells—eosinophils, basophils, and regulatory T lymphocytes—as well as its structural and functional similarities to other galectins is, however, indicative of a potentially crucial role in inflammation. This review presents a brief description of Charcot-Leyden crystal protein—examining what is currently known about its association with two major immuno-regulatory cells, eosinophils and regulatory T lymphocytes, and its potential intracellular and extracellular function in eosinophil pre-mRNA splicing and the modulation of eosinophil-T lymphocyte interactions, respectively.

## Eosinophilic charcot-leyden crystal protein

Charcot-Leyden crystal protein is a major constituent of eosinophils (and basophils) [9] and a hallmark of eosinophil-associated inflammatory reactions [10-12]. The crystals are distinct, colorless, hexagonal and bi-pyramidal; 20µm to 40µm in length, and 2 µm to 4µm across [13,14]. They are unique to primate eosinophils [15] and are frequently observed in human tissues and secretions in association with eosinophilic inflammatory responses, such as asthma, myeloid leukemias, allergic and parasitic diseases [11,16], and various types of cancers [17-19] (Figure 1). The protein is among the most abundant of eosinophil constituents [11,20], comprising an estimated 7% to 10% of total eosinophil cellular protein [21]—an amount comparable with the eosinophil's content of toxic cationic proteins and enzymes, such as MBP and EPO [10]. The 16.5 kDa hydrophobic protein [20], localized primarily to a small cytoplasmic granule fraction [9] and to the nucleus of eosinophils [10], lacks a secretion signal peptide and transmembrane domain, and is secreted under certain conditions by nonclassical and novel apocrine mechanisms [4]. Eosinophil development and Ca<sup>2+</sup> ionophores stimulate its secretion [22]; and the

CLC gene, localized on chromosome 19 [13], is transcriptionally induced by butyric acid [23].



**Figure 1: Photomicrograph of Charcot-Leyden Crystals in Prostatic Adenocarcinoma.** A transrectal needle biopsy of the prostate shows an adenocarcinoma and numerous degranulated eosinophils in the peritumor fibrous stroma. An intraepithelial group of eosinophils is seen (thick arrow) and intraluminal Charcot-Leyden crystals (thin arrow). Photomicrograph reproduced from *Diagnostic Pathology* 2006, 1:26 [9].

The protein was first identified in 1853 by Charcot and Robin, who described needle-shaped, crystalline deposits of the protein in the spleen and heart blood of a leukemic patient [13,24], and later in 1872 by Leyden in his studies of the deposits in the sputum of asthmatics [25]; hence, their designation as Charcot-Leyden crystals. Although initially identified as the eosinophil's premier lysophospholipase [9], studies have shown significant amino acid sequence homology with galectins [26], a multi-protein superfamily of galactoside-binding proteins, with characterized roles in inflammation, tumor surveillance [27], autoimmunity, infections, and allergic reactions [4]. Unlike its  $\beta$ -galactoside-binding counterparts, crystallographic studies have shown selective recognition of mannose by the eosinophil Charcot-Leyden crystal protein [28]. Among all the galectins, CLC is most structurally similar to galectins-1 and -2 and its lectin/carbohydrate-recognition domain is most sequentially similar to galectin-3 [10]. Studies have shown that CLC interacts with eosinophil toxic cationic proteins, ECP and EDN, and may be involved in their secretion [29]. Despite the established association between CLC and eosinophil-associated inflammation, its biochemical and functional properties remain indeterminate [20].

### Charcot-leyden crystals in regulatory T cells

In addition to localization in eosinophils (and basophils), recent studies have reported intra-cytoplasmic CLC expression in human  $CD4^+ CD25^+ Foxp3^+$  regulatory T cells (Treg) [30]. Its expression is not only necessary for limiting Treg-proliferation and maintaining Treg anergy, but is also crucial for maintaining Treg-mediated immuno-suppression of co-cultured  $CD4^+$  T lymphocytes [30]. The protein is constitutively expressed in the Tregs and is not secreted nor released under the described experimental conditions [30]. Regulatory

T cells play a crucial role in the maintenance of immunological self-tolerance [30,31], the prevention of a variety of autoimmune and inflammatory diseases, and inhibit antitumor immune reactions by suppressing tumor-specific T cell responses [31]. Although little is known about the molecular mechanisms and proteins that contribute to the *in vitro* anergy and regulatory activity of Tregs [30], these findings offer implications for Charcot-Leyden crystal protein in their functional properties.

### Conclusions

The identification of Charcot-Leyden crystals at sites of eosinophil infiltration in tissues and secretions has been, for many years, a marker of eosinophil involvement in inflammatory reactions. Eosinophils are immuno-modulatory cells that function as proinflammatory and cytotoxic-effector cells in inflammation. What exactly do these findings reveal about the biochemical properties and functional role(s) of the distinctive crystals in eosinophil biology? CLC is among the eosinophil's most abundant proteins; and, the fact that these immune cells contain such large quantities, offers implications for a significant role in eosinophil biology and function. CLC, like all galectins, lacks a secretory signal peptide [4]. While the absence of a signal peptide, as well as its nuclear and cytosolic localizations, are suggestive of a strictly intracellular function, its atypical secretion or exportation, however, strongly suggests a potential extracellular role(s) in eosinophil function [10]. Galectin-3 has been identified as a nuclear factor in the regulation of pre-mRNA splicing [32]. CLC's similarity to galectin-3, therefore, gives credence to the intriguing possibility of CLC's intracellular function(s) in pre-mRNA splicing [10]. Galectin-3's IgE [33] and carbohydrate (laminin)-binding [34] abilities also provoke the idea of an extracellular role(s) for CLC in eosinophil function. Equally intriguing is the modulatory role of the protein in regulatory T lymphocytes. Eosinophils, like Tregs, possess immuno-regulatory properties [35] and regulate a variety of immune cells. They express surface antigens, as well as produce and release most cytokines that regulate and promote T-cell activation, proliferation, and cytokine secretion [2]. Given CLC's necessity for Treg suppression of  $CD4^+$  T lymphocytes, one can certainly envision a role for eosinophilic CLC in regulating the proliferation and function of  $CD4^+$  T cells.

Charcot-Leyden crystals represent active legacies of eosinophil infiltration in peripheral tissues. The protein is more than a curious crystalline artefact—persisting in tissues long after eosinophil death. Given the findings presented here, and the multifunctional nature of galectins, the role of galectin-10/CLC in eosinophil biology and function seems worthy of further exploration.

### Acknowledgement

This review is part of a study on eosinophils in health and disease funded by the Washington Baltimore Hampton Roads Louis Stokes Alliance for Minority Participation (WBHR-LSAMP)- NSF Grant Number HRD-1000286

### References

1. Melo RC, Spencer LA, Dvorak AM, Weller PF (2008) Mechanisms of eosinophil secretion: large vesiculotubular carriers mediate transport and release of granule-derived cytokines and other proteins. *J Leukoc Biol* 83: 229-236.
2. Davis BP, Rothenberg ME (2014) Eosinophils and cancer. *Cancer Immunol Res* 2: 1-8.

3. Anatole AK, Peter GT (2012) Galectins in Disease and Potential Therapeutic Approaches. Galectins and Disease Implications for Targeted Therapeutics. ACS Symposium Series: American Chemical Society.
4. Rabinovich GA, Rubinstein N, Fainboim L (2002) Unlocking the secrets of galectins: a challenge at the frontier of glyco-immunology. *J Leukoc Biol* 71: 741-752.
5. Fujihara S, Mori H, Kobara H, Rafiq K, Niki T, et al. (2013) Galectin-9 in cancer therapy. *Recent Pat Endocr Metab Immune Drug Discov* 7: 130-137.
6. Pena C, Mirandola L, Figueroa JA, Hosiriluck N, Suvorava N, et al. (2014) Galectins as therapeutic targets for hematological malignancies: a hopeful sweetness. *Ann Transl Med* 2: 87.
7. Wiersma VR, de Bruyn M, Helfrich W, Bremer E (2013) Therapeutic potential of Galectin-9 in human disease. *Med Res Rev* 33 Suppl 1: E102-126.
8. Salatino M, Croci DO, Bianco GA, Ilarregui JM, Toscano MA, et al. (2008) Galectin-1 as a potential therapeutic target in autoimmune disorders and cancer. *Expert Opin Biol Ther* 8: 45-57.
9. Aydin O (2006) Charcot-Leyden crystals in a prostatic adenocarcinoma. *Diagn Pathol* 1: 26.
10. Dvorak AM, Letourneau L, Login GR, Weller PF, Ackerman SJ (1988) Ultrastructural localization of the Charcot-Leyden crystal protein (lysophospholipase) to a distinct crystalloid-free granule population in mature human eosinophils. *Blood* 72: 150-158.
11. Leonidas DD, Elbert BL, Zhou Z, Leffler H, Ackerman SJ, et al. (1995) Crystal structure of human Charcot-Leyden crystal protein, an eosinophil lysophospholipase, identifies it as a new member of the carbohydrate-binding family of galectins. *Structure* 3: 1379-1393.
12. Ackerman SJ, Weil GJ, Gleich GJ (1982) Formation of Charcot-Leyden crystals by human basophils. *J Exp Med* 155: 1597-1609.
13. Dor PJ, Ackerman SJ, Gleich GJ (1984) Charcot-Leyden crystal protein and eosinophil granule major basic protein in sputum of patients with respiratory diseases. *Am Rev Respir Dis* 130: 1072-1077.
14. Giembycz MA, Lindsay MA (1999) Pharmacology of the eosinophil. *Pharmacol Rev* 51: 213-340.
15. Zucker-Franklin D (1980) Eosinophil structure and maturation. *The eosinophil in health and disease* 43.
16. El-Hashimi W (1971) Charcot-Leyden crystals. Formation from primate and lack of formation from nonprimate eosinophils. *Am J Pathol* 65: 311-324.
17. Thompson JH, Paddock FK (1940) The Significance of Charcot-Leyden Crystals. *New England Journal of Medicine* 223:936-939.
18. Barrow H, Rhodes JM, Yu LG (2011) The role of galectins in colorectal cancer progression. *Int J Cancer* 129: 1-8.
19. Liu FT, Rabinovich GA (2005) Galectins as modulators of tumour progression. *Nat Rev Cancer* 5: 29-41.
20. Newlaczyl AU, Yu LG (2011) Galectin-3--a jack-of-all-trades in cancer. *Cancer Lett* 313: 123-128.
21. Ackerman SJ, Loegering DA, Gleich GJ (1980) The human eosinophil Charcot-Leyden crystal protein: biochemical characteristics and measurement by radioimmunoassay. *J Immunol* 125: 2118-2126.
22. Weller PF, Bach DS, Austen KF (1984) Biochemical characterization of human eosinophil Charcot-Leyden crystal protein (lysophospholipase). *J Biol Chem* 259: 15100-15105.
23. Butterfield JH, Ackerman SJ, Scott RE, Pierre RV, Gleich GJ (1984) Evidence for secretion of human eosinophil granule major basic protein and Charcot-Leyden crystal protein during eosinophil maturation. *Exp Hematol* 12: 163-170.
24. Dyer KD, Rosenberg HF (2001) Transcriptional regulation of galectin-10 (eosinophil Charcot-Leyden crystal protein): a GC box (-44 to -50) controls butyric acid induction of gene expression. *Life Sci* 69: 201-212.
25. Charcot J, Robin C (1853) Observation de leucocythemie. *CR Mem Soc Biol* 5:454-450.
26. Spry CJF (1988) Eosinophils: A Comprehensive Review, and Guide to the Scientific and Medical Literature. Oxford University Press.
27. Ackerman SJ, Corrette SE, Rosenberg HF, Bennett JC, Mastrianni DM, et al. (1993) Molecular cloning and characterization of human eosinophil Charcot-Leyden crystal protein (lysophospholipase). Similarities to IgE binding proteins and the S-type animal lectin superfamily. *J Immunol* 150: 456-468.
28. Rosenberg HF (2007) Suppression, surprise: galectin-10 and Treg cells. *Blood* 110:1407-1408.
29. Swaminathan GJ1, Leonidas DD, Savage MP, Ackerman SJ, Acharya KR (1999) Selective recognition of mannose by the human eosinophil Charcot-Leyden crystal protein (galectin-10): a crystallographic study at 1.8 Å resolution. *Biochemistry* 38: 13837-13843.
30. Doyle CB, Ackerman SJ (2009) Lentiviral shRNA knockdown of Charcot-Leyden Crystal Protein/Galectin-10 Expression in Developing Human Eosinophils Impairs Granulogenesis. *Journal of Allergy and Clinical Immunology* 123:S270.
31. Kubach J, Lutter P, Bopp T, Stoll S, Becker C, et al. (2007) Human CD4+CD25+ regulatory T cells: proteome analysis identifies galectin-10 as a novel marker essential for their energy and suppressive function. *Blood* 110: 1550-1558.
32. Jonuleit H, Schmitt E (2005) Regulatory T-cells in antitumor therapy: isolation and functional testing of CD4+CD25+ regulatory T-cells. *Methods Mol Med* 109: 285-296.
33. Dagher SF, Wang JL, Patterson RJ (1995) Identification of galectin-3 as a factor in pre-mRNA splicing. *Proc Natl Acad Sci U S A* 92: 1213-1217.
34. Zuberi R, Frigeri LG, Liu FT (1994) Activation of rat basophilic leukemia cells by epsilon BP, an IgE-binding endogenous lectin. *Cell Immunol* 156: 1-12.
35. Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, et al. (1994) Galectins: a family of animal beta-galactoside-binding lectins. *Cell* 76: 597-598.
36. Jacobsen EA, Taranova AG, Lee NA, Lee JJ (2007) Eosinophils: singularly destructive effector cells or purveyors of immunoregulation? *J Allergy Clin Immunol* 119: 1313-1320.