ORIGINAL RESEARCH

Atopy and HIV: Do basophils play a role in both?

Investigating basophil counts and activation marker expression in different disease states

Sara Naimimohasses *(Fourth Year Medicine, TCD)*

Clinical Points

- HIV positive patients had statistically significant lower absolute basophil counts and higher levels of CD63 expression *in vivo* compared to healthy controls.
- Absolute basophil counts in the eczema, asthma and allergy patient groups did not statistically differ from the healthy controls.
- There was no statistically significant variation in basophil CD63 expression in the eczema, allergy and asthma patient groups in comparison with healthy controls.
- This study is one of the first to compare absolute basophil counts alongside *in vivo* CD63 expression. The majority of other studies have focused on CD63 expression following allergen challenge *in vitro*.
- Future research directives may focus on the role of basophils in different stages of HIV infection.

Abstract

Background: Recent experimental evidence has implicated a role for basophils in allergic diseases. Basophils are also believed to be stimulated in HIV-1 infection by the glycoprotein expressed on the surface of the viral envelope, gp120, and directly through viral interaction with the chemokine receptor CCR3.

Objectives: To investigate absolute basophil counts and peripheral blood basophil surface marker expression in healthy individuals, patients with atopic diseases and HIV positive patients.

Methods: Blood was taken from a total of 68 patients: 17 healthy adult volunteers, 18 patients diagnosed with asthma, four patients with eczema, 14 patients with a suggestive history of allergy and 15 HIV positive patients. The samples were stained with anti-CD123, anti-HLA-DR and anti-CD63 antibodies. A double gating strategy was used to isolate the basophil population and analyse CD63 expression.

Results: No significant difference was found between the absolute basophil count in patients with asthma (*p=0.402*) and eczema (*p=0.947*) compared to the healthy volunteers. HIV positive patients (*p=0.007*) and allergic patients (*p=0.022*) had statistically significant lower basophil counts compared to healthy controls. No significant difference was found in the level of CD63 expression in asthma patients (*p=0.521*), eczema patients (*p=0.288*) and patients with allergies (*p=0.346*). HIV positive patients expressed significantly higher levels of CD63 compared to healthy volunteers (*p=0.005*).

Conclusion: There was a significant reduction in absolute basophil counts in patients with HIV, which may be due to the virus directly infecting basophils, reducing their $T_{1/2}$ in circulation. The reduction in basophil count seen in allergic patients could be explained by allergen-induced migration. Basophils from HIV positive individuals expressed significantly higher levels of CD63, possibly owing to the allergen-like function of HIV gp120. There was no evidence to suggest patients with atopic diseases expressed higher levels of CD63.

Introduction

Over the past few decades there has been a progressive increase in the incidence of atopic diseases. More than 20% of the world population is affected by IgE-mediated allergic diseases, with an estimated 300 million people alone suffering from allergic asthma[1,21,31].

The central pathophysiology behind allergic diseases is believed to be an imbalance in the expression of two $CD4^+$ T lymphocyte subsets: Th1 and Th2. Th1 lymphocytes secrete IL-2, IFN-α and TNF-β, which activate macrophages and are involved in stimulating cell-mediated immunity whilst simultaneously inhibiting humoral immunity. Th2 lymphocytes secrete IL-4 and IL-13 and are involved in activating the humoral immune response by promoting B cell proliferation and heavy chain class switching, stimulating allergen-specific IgE production. It has been hypothesised that abnormal polarization of the immune system from an early age leading to an increased Th2:Th1 ratio could result in an atopic phenotype[14,40].

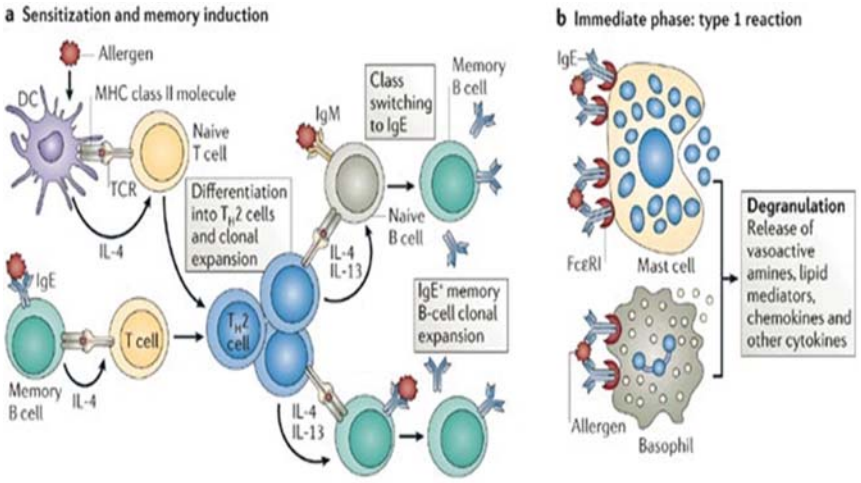
IgE-mediated hypersensitivity is thought to be triggered by a sequence of events following initial allergen exposure. Specialised antigen presenting cells, such as macrophages and dendritic cells, process and present allergens via cell surface MHC class II molecules to allergen-specific Th2 cells. This activates allergen-specific Th2 cells, causing them to release IL-4 and IL-13. The secretion of IL-4 and IL-13, combined with T cell CD40 ligand-CD40 receptor interaction, facilitates B cell proliferation, differentiation and heavy-chain isotype class switching to the IgE subtype. The activated B cells

produce and secrete allergen-specific IgE antibodies, which bind to FCεRI receptors on basophils and mast cells. Subsequent allergen exposure cross-links the IgE molecules and leads to activation, basophil degranulation, the release of vasoactive amines and lipid mediators and the synthesis of cytokines[17, 21, 22, 26](Figure 1). These mediators stimulate vasodilatation, increase vascular permeability, activate the complement cascade and cause migration of neutrophils, mast cells and basophils. This leads to the clinical manifestation of allergic diseases such as urticaria, angioedema and anaphylaxis.

Recent experimental evidence has demonstrated the pathogenic role of basophils in IgE-mediated hypersensitivity[8,10,12,14,17,20]. Basophils have been found in bronchial biopsies from asthmatic patients, in nasal lavage fluids following allergen provocation in patients with allergic rhinitis and in skin biopsies from patients with atopic dermatitis[14,30,37].

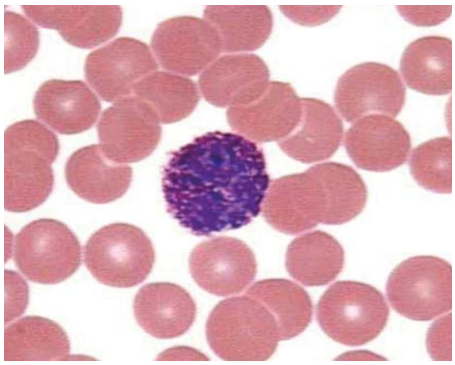
Basophils are small, circulating leukocytes with cytoplasmic granules that stain metachromatically with basic dyes (Figure 2)[32]. They constitute less than 0.2% of peripheral blood leukocytes and are only recruited in peripheral tissue in disease states[3,6]. They are derived from $CD34^+$ haematopoietic progenitor cells, which differentiate and mature in the bone marrow in the presence of IL-3, and have a lifespan of several days[12,13,19].

The cellular source of the early peak in IL-4 responsible for triggering the Th2-type immune response has been the subject of much debate; however, basophils have recently been identified as the main source of the cytokine. IL-4 stimulates the differentiation of naïve $CD4^+$ T cells to the Th2 type; basophils are consequently thought to act



▲ **Figure 1.** A, Allergen induces the humorally-mediated immune response and produces Th2 cells, which in turn encourage B cells to secrete IgE antibodies. B, Allergen activates mast cells and basophils, causing degranulation of substances that cause the clinical features of allergic disorders[47]. Taken from Nature Review Immunology.

as modulators of the immune response. Following activation, they release large quantities of the pro-inflammatory mediators histamine and leukotriene C4 and rapidly synthesise the Th2-type cytokines IL-4 and IL-13[3,6,12,16]. Release of these cytokines combined with T-cell -CD40 ligand interaction promotes B cell proliferation and heavy chain

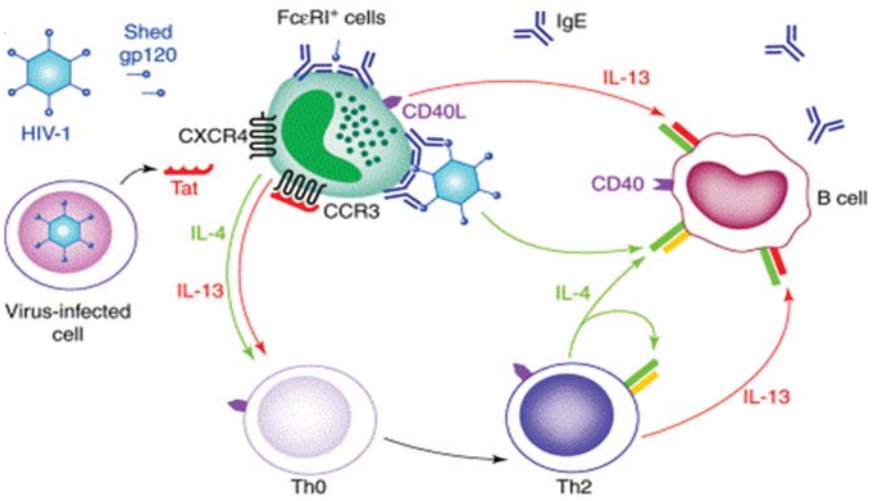


▲ **Figure 2.** Peripheral blood film showing basophil in centre, which stains blue due to the negatively charged cytoplasmic granules[44].

isotype switching to the IgE and IgG4 subtypes. The cytokines also play an important role in leukocyte recruitment to affected tissues by increasing expression of the cell adhesion molecule VCAM-1 in endothelial cells and synthesis of the chemokine eotaxin[3, 6, 15, 27].

What is the link between HIV infection and allergic diseases? Although there is no direct relationship between the two, studies have shown that HIV-1 infection may influence the behaviour of basophils by polarising the host immune response to be humorally-mediated[24,25].This may explain why HIV positive patients demonstrate increased prevalence and severity of allergic reactions[33, 36].

Previous studies on HIV-1 pathogenesis showed a shift to the Th2-type immune response, an increase in serum IgE levels and increased IL-4 and IL-13 in patients' lymph nodes. This indicates a possible allergen-like function executed by HIV-1[24, 25, 33]. The basophil chemokine receptor CCR3 also serves as a co-receptor through which HIV-1 particle can directly infect the basophil[28]. Further investigation has revealed that the HIV-1 glycoprotein gp120 contains a superantigen domain, which binds to the VH3 region of VH3+ IgE molecules bound to the FCεRI on basophils and mast cells. This gp120 - VH3 domain interaction resembles that of an allergen,



▲ **Figure 3. FCεRI positive cells express CCR3 and CXCR4 which act as co-receptors for specific strains of HIV-1 can serve as direct routes for HIV-1 infection. Shed or bound viral gp 120 also binds to the VH3+ domain of surface bound IgE molecules, stimulating the release of IL-4 and IL-13. The viral protein Tat functions as a virokine through its interaction with the chemokine receptor, CCR3 on FCεRI positive cells, stimulating the migration of basophils and mast cells to the site of HIV-1 infection[45].**

stimulating the basophil and leading to degranulation. This is an important mechanism by which the virus modulates the immune response to the Th2-type, inhibiting the host's adaptive cell-mediated immunity vital in killing HIV infected cells, whilst simultaneously increasing the pool of cells susceptible to infection[23,25,28].

Another product of the HIV-1 virus, Tat protein, increases the accessibility of basophils and mast cells by acting as a virokine[33]. Tat protein is released by HIV-1 infected cells and stimulates the migration of basophils and mast cells to the site of HIV-1 infection through its interaction with the chemokine receptor CCR3 expressed on the surface of basophils. In addition, Tat stimulates the up-regulation of CCR3 receptors, further facilitating HIV-1 infection of FCεRI positive cells[33,34](Figure 3).

How can the peripheral basophil population and activation marker expression be analyzed? Basophils can be identified by labelling the cells with antibodies conjugated with fluorochromes. Laser light excites the fluorochromes and this causes them to emit light at a specific wavelength. The cells themselves also scatter light according to their size and cytoplasmic complexity. The light emitted and scattered is then detected by photomultipliers and is processed by the computer enabling specific cell populations to be analyzed through a variety of different parameters.

Basophils constitutively express high levels of the IL-3 receptor α chain, CD123. CD123 is a member of the type 1 cytokine receptor family with a single transmembrane-spanning segment. It is a low affinity IL-3 receptor and its stimulation encourages cell proliferation and differentiation. This receptor is also expressed on CD34+ cells, monocytes, neutrophils and plasmacytoid dendritic cells. The use of monoclonal antibodies against the α chain of the CD123 predominantly stains basophils and dendritic cells. This enables accurate differentiation from neutrophils and monocytes in a side scatter versus CD123 expression graph, otherwise known as a 'dot plot'. Basophils express very low levels of HLA-DR, which can be used to differentiate them from dendritic cells[7,29].

Quantifying the expression of the glycoprotein CD63 on the plasma membrane of basophils can be used as a measure of basophil activation. CD63 is expressed on mast cells, macrophages, eosinophils and platelets. It is usually found within the cell attached to intracytoplasmic granules. Following stimulation, degranulation leads to the fusion of these granules with the plasma membrane and their subsequent expression on the surface of the cell[2,4,5]. It is believed that CD63 mediates signal transduction events involved in cell development, activation and motility, although its precise function in basophils is unknown. Previous studies have shown that CD63 expression mirrors basophil histamine release, which demonstrates that it is a reliable method of evaluating basophil activation[2,4,5,11, 13,20].

Previously, the lack of basophil-specific markers and the difficulties in purifying techniques meant that very little was understood about the function of basophils; however, recent development of specific monoclonal antibodies has enabled basophil enumeration and identification in tissues, shedding further light on their role. To date, CD63 expression has been used to analyse allergen-specific activation of basophils *in vitro*[2,10,12]; however, few studies have compared the resting *in vivo* basophil CD63 expression of individuals with atopic diseases and HIV infection to those of healthy controls[20,37]. This paper aims to explore if there is a difference in basophil number and activation marker expression in atopy and HIV infection compared with healthy controls, helping to further understand their behaviour *in vivo*.

METHODS

In this study, approved by the institutional ethics committee, blood was taken from 17 healthy adult volunteers who were HIV negative and had no history of atopy (7 females, 11 males, mean age=38.8, range 23-58). Samples were taken from 18 patients diagnosed with asthma (13 females, 5 males, mean age=54.9, range 30-80), four patients diagnosed with eczema (3 females, 1 male, mean age=44.3, range 22-73) and 14 patients with a suggestive history of allergy (10 females, 4 males, mean age=43.1, range 20-68). Blood was taken from 15 HIV positive patients and was analysed (1 female, 14 males, mean age=37.7, range 22-45). All participants gave informed consent. Patients had previously been diagnosed and were attending dermatology, respiratory, immunology and infectious diseases outpatient clinics for their conditions. Patients remained on treatment throughout the study. Inclusion criteria for the allergy subgroup consisted of a history of urticaria, angioedema or anaphylaxis. For the asthma and eczema subgroups, we set out to include patients with a history of childhood onset only; however, in practice some of the samples obtained were from patients who suffered from intrinsic asthma. Patients with serological evidence of HIV infection were included in the HIV positive subgroup. No specific exclusion criteria were defined for the study.

100 µL of venous whole blood collected in EDTA-coated tubes was incubated with antibodies (5 µL PE-CD123, 5 µL APC AntiHLA-DR, 5 µL Anti-human CD63) for 15 minutes at room temperature in the dark. 2ml of LysingSolution ® was then added to lyse the erythrocytes. The samples were then centrifuged at room temperature at 1200 rpm for 5 minutes. The supernatant was discarded and the cells were washed in 2mls of FACSflow. The samples were re-centrifuged at 1200 rpm for 5 minutes, the supernatant discarded, and the cells were suspended in 0.5mls of Cell FIX.

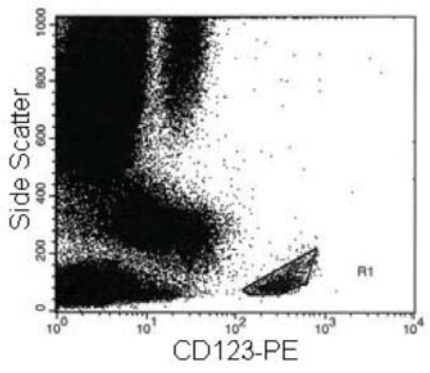
The data was then acquired using a FACSCalibur and analyzed using Cell Quest Pro software® (BD Biosciences). A double gating strategy was employed. Initially, a dot plot of side scatter versus CD123 expression (Figure 4) was drawn up to gate on cell populations with relatively high CD123 expression. The basophil population was then identified as displaying relatively high levels of CD123 expression coupled with low HLA-DR expression (Figure 5). At least 1000 basophils were gated on. Absolute basophil count was calculated using the percentage of total cells acquired that were identified as basophils and the total white cell count.

Absolute basophil count = (Percentage total x White cell count)/100

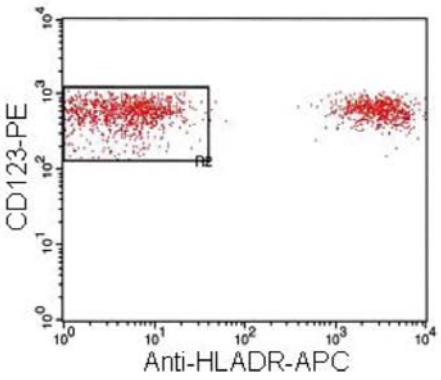
The gated basophil population was then analysed using a biparametric dot plot of CD123 expression against CD63 expression (Figure 6). Basophil population CD63 expression was measured as the geometric mean fluorescence intensity detected for CD63-FITC. A reference range for CD63 expression was established using values from the volunteers: 24.19-13.71 (mean: 18.95, SD: 2.62, Range: 22.90- 14.76).

The protocol employed to identify the basophil population and CD63 expression using flow cytometry was similar to that used by Gyimesi *et al* [9].

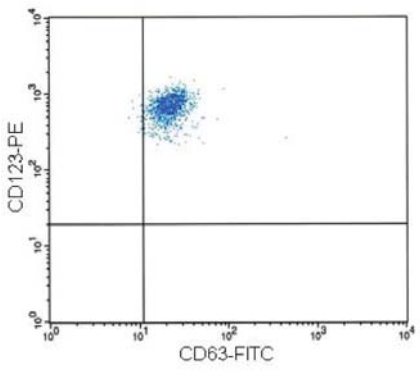
Statistical analysis was performed by Microsoft Excel.



▲ **Figure 4.** Cell populations with high levels of CD123-PE are gated on; region consists of basophils, monocytes and dendritic cells.



▲ **Figure 5.** The basophil population is gated on; high CD123 expression coupled with low HLA-DR expression.



▲ **Figure 6.** Basophils in the upper right quadrant stain positive for CD63.

	Healthy Controls	Asthma Patients	Allergy Patients	Eczema Patients	HIV Patients
Number of Patients:	17	18	11	4	15
Mean absolute basophil no: x10³cells /μL	0.0515	0.0453	0.0321	0.0507	0.0297
SD:	0.0222	0.0214	0.0151	0.0075	0.0203
Range:	0.016-0.099	0.0141-0.0955	0.0137-0.0639	0.0415-0.0584	0.0069-0.0655

Table 1: A table displaying the mean, standard deviation (SD) and range of absolute basophil counts in the different patient groups.

	Healthy Controls	Asthma Patients	Allergy Patients	Eczema Patients	HIV Patients
Number of Patients:	17	18	14	4	15
Mean CD63 expression: Mean fluorescence intensity (MFI)	18.95	18.27	20.31	17.42	23.41
SD:	2.62	3.52	5.09	2.01	5.41
Range:	22.90-14.76	26.38-12.78	29.77-13.39	20.33-15.79	30.29-15.51

Table 2: A table displaying the mean, standard deviation (SD) and range of basophil CD63 expression measured as mean fluorescence intensity in the different patient groups.

RESULTS

Table 1 compares the absolute basophil count between the different patient groups. Full blood counts for three of the allergy patients were unavailable; consequently, only the results of 11 allergic patients were available for comparison.

HIV positive patients ($p=0.007$) and allergic patients ($p=0.022$) had statistically significant lower basophil counts compared to the healthy controls. No significant difference was found between the absolute basophil count in patients with asthma ($p=0.402$) or eczema ($p=0.947$) compared to healthy controls (Figure 7).

On analysing the results from the healthy volunteers, a large proportion of basophils (mean: 93.81%, range: 100.00-76.26%, SD: 5.85) were present in the positive quadrant, which indicates that the majority weakly expressed CD63. No significant difference was found between the percentages of basophils present in the positive quadrant in healthy controls compared to asthma patients ($p=0.489$), allergy patients ($p=0.254$), eczema patients ($p=0.251$) or HIV patients ($p=0.471$) (Table 2).

HIV positive patients expressed significantly higher levels of CD63 compared to the healthy controls ($p=0.005$). No significant difference was found in the level of CD63 expression in asthma patients ($p=0.521$), eczema patients ($p=0.288$) or patients with allergies ($p=0.346$) compared to healthy controls (Figure 8).

There was no significant difference in the level of CD63 expression in atopic individuals (serum IgE levels >120 kU/L) in comparison to non-atopic individuals ($p=0.919$) (Figure 9).

The results also showed no significant difference in the level of CD63 expression between males and females in the control group ($p=0.593$).

Figure 7. A graph comparing absolute basophil counts in the different patient groups. There was no significant difference between CD63 expression in patients with eczema, asthma compared to the healthy volunteers. There was a significant decrease in the basophil counts of HIV patients and patients with allergies.

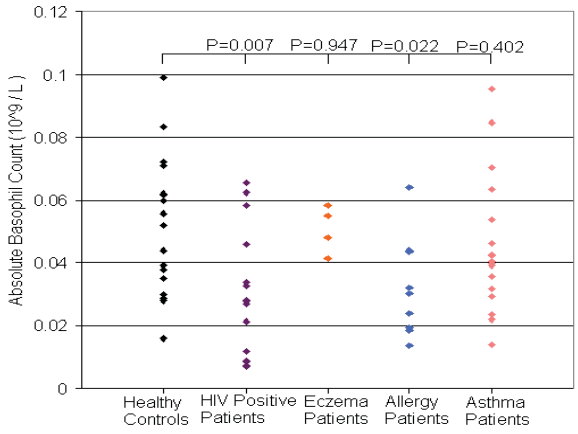
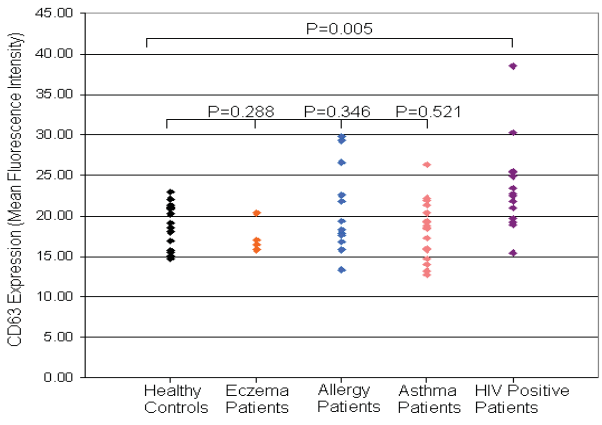
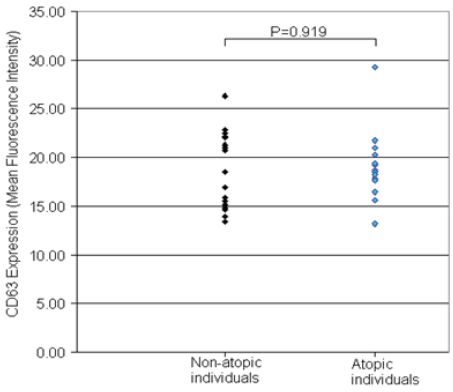


Figure 8. A graph comparing the level of CD63 expression in the different patient groups. There was no significant difference between CD63 expression in patients with eczema, asthma and allergies compared to the healthy volunteers. There was a significant increase in CD63 expression in HIV patients





▲ **Figure 9.** **This graph compares the level of CD63 expression in non-atopic individuals and atopic individuals.**

Discussion

Basophil counts were reduced in allergic patients. This may be due to allergen-induced basophil migration into the affected tissues uncompensated by release from the bone marrow, reducing the numbers in circulation. Since a relatively small patient sample was used, it is necessary to repeat the investigation with larger patient groups to determine the significance of this apparent reduction. It would be valuable to elucidate the relationship, if any, between different allergic reactions, such as urticaria, angioedema and anaphylaxis, and absolute basophil counts.

There was a statistically significant reduction in absolute basophil counts in patients with HIV. Basophils express CCR3, which binds to the chemokines eotaxin and RANTES, mediating signal transduction events necessary for migration. CCR3 also functions as a co-receptor for HIV infection. Consequently, a fraction of basophils in HIV positive patients are directly infected by the virus, significantly reducing their half-life in circulation. Basophil maturation and release may be unable to compensate for the decreased half-life of HIV-infected basophils. Another suggestion for the observed decrease in basophil counts may be due to Tat-mediated basophil migration to HIV-1 infected tissue, thereby decreasing basophils in systemic circulation. All HIV positive individuals who took part in this study were on anti-retroviral therapy, it would be interesting to investigate whether antiretroviral drugs have any effect on peripheral blood basophil counts and activation marker expression.

No significant difference was found in the absolute basophil counts in patients with asthma or eczema as compared to healthy volunteers. This could suggest that basophil migration from systemic circulation to the affected tissues in these conditions is balanced by basophil maturation and release into peripheral blood.

The results obtained from this experiment revealed no significant difference in the level of basophil CD63 expression in asthmatic patients. This could be explained by the fact that most of the patients who took part in the study were on treatment with steroids. Steroids have been shown to cause a reduction in absolute basophil count and inhibit mediator release[38]. It would, therefore, be recommended to exclude patients on regular systemic steroid therapy from future investigations.

No significant difference was found in the level of CD63 expression in allergic patients. It is important to note that a heterogeneous patient group was selected. Some individuals who reported allergic reactions to unspecified allergens may have suffered from idiopathic urticaria or angioedema. The underlying mechanism may be autoimmune in nature, mediated by auto-antibodies directed against the FCεRI receptors.

The eczema patient group was too small to allow for effective analysis and comparison. Nevertheless, statistical analysis showed no significant difference in CD63 expression. Two of the patients were on oral steroids and one was on immunosuppressant therapy, which might explain the results obtained.

The site of allergen exposure determines the organ systems affected. In patients with asthma, eczema and allergies, the allergens remain localised to the tissues affected. Basophils have been shown to migrate to those tissues in those conditions[14,18,37]. Consequently, the results could demonstrate that basophils in peripheral blood are not an accurate representation of those directly involved in the pathogenesis of the conditions. Basophils that have migrated to the affected regions are exposed to the allergen and are more likely to be stimulated and may consequently express higher levels of CD63 than those present in circulation.

This study demonstrated a significant increase in basophil CD63 expression in HIV patients despite a significant reduction in absolute basophil counts compared to the healthy controls. This could suggest that a greater proportion of basophils present in peripheral blood were stimulated in HIV in comparison to healthy controls. The HIV glycoprotein, gp120, is a viral envelope protein. It is present in systemic circulation as either virus-bound or in its shed form and its concentration in circulation increases as the virus replicates. In the early stages of HIV infection associated with viraemia, a rise in serum IgE and IL-4 has been clinically observed[23,24,25,33]. Since the glycoprotein is not localised to specific tissues, peripheral blood basophils are exposed to the superantigen and are stimulated via

its interaction with the VH3 domain of the VH3+ IgE molecules[23,24,25]. This may lead to the production of the Th2 type cytokines IL-4 and IL-13 and the up-regulation of CD63 on the cell surface. HIV preferentially replicates in Th2 cells. Hence, HIV-1 induced basophil activation can be considered a method by which the virus optimises conditions for replication. Early studies have shown that peripheral IgE levels may serve as a marker for poor prognosis in HIV positive individuals[39]. It would be interesting to see if there is a relationship between a history of atopy and disease severity following infection. It is also a potentially exciting avenue to explore with regards to novel medical interventions. Could inhibition of HIV-induced modulation of the immune response in early stage HIV infection serve as a viable therapeutic option?

Conclusion

The results of this study showed no difference in absolute basophil counts in patients with asthma or eczema compared to healthy controls. There was a significant reduction in the basophil counts in patients with allergies and HIV positive patients. There was no evidence to suggest that peripheral blood basophils from patients with atopic diseases expressed higher levels of the basophil activation marker CD63 compared to healthy individuals. Basophils from HIV positive individuals expressed significantly higher levels of CD63, possibly owing to the allergen-like function of the viral envelop protein, gp120. Further studies should investigate basophil CD63 expression in HIV positive patients at different stages in disease progression and assess absolute basophil numbers in better characterised allergic patient groups.

With special thanks to: Professor Feighery, Dr John Jackson, Dr Niall Conlon and all the staff of the Immunology department at St. James' Hospital, Dublin. Thanks to the Health Research Board for funding this summer research project.

References

1. World Health Organization. Chronic respiratory diseases. [Online]. 2005 [cited 2009 Dec 31]; Available from: URL:http://www.who.int/respiratory/asthma/en/
2. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, DeClerck LS, Stevens WJ. In vitro allergy diagnosis: should we follow the flow? Clin Exp Allergy 2004;34:332-9.
3. Gibbs BF. Human basophils as effectors and immunomodulators of allergic inflammation and innate immunity. Clin Exp Med 2005;5:43-9.
4. Frezzolini A, Provini A, Teofoli P, Pomponi D, DePita O. Serum induced basophil CD63 expression by means of a tricolour flow cytometric method for in vitro diagnosis of chronic urticaria. Allergy 2006;61:1071-7.
5. Ocmant A, Peignois Y, Mulier S, Hanssens L, Michils A, Schanene L. Flow cytometry for basophil activation markers: the measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. J Immunol Methods 2007;320:40-8.
6. Falcone FH, Zillikens D, Gibbs BF. The 21st century renaissance of the basophil? Current insights into its role in allergic responses and innate immunity. Exp Dermatol 2006;15:855-64.
7. Ducrest S, Meier F, Tschopp C, Pavlovic R, Dahinden CA. Flow cytometric analysis of basophil counts in human blood and inaccuracy of haematology analyzers. Allergy 2005;60:1446-50.
8. Sanz ML, Sanchez G, Gamboa PM, Vila L, Uasuf C, Chazot M, et al. Allergen induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. Clin Exp Allergy 2001;21:1007-13.
9. Gyimesi E, Sipka S, Danko K, Kiss E, Hidvegi B, Gal M, et al. Basophil CD63 expression assay on highly sensitized atopic donor leucocytes–a useful method in diagnosing chronic autoimmune urticaria. Br J Dermatol 2004;151:388-96.
10. Abuaf N, Rajoely B, Ghazouani E, Lev DA, Pecquet C, Chabane H, et al. Validation of a flow cytometric assay detecting in vitro basophil activation for the diagnosis of muscle relaxant allergy. J Allergy Clin Immunol 1999;104:411-8.
11. Boumiza R, Debard AL, Monneret G. The basophil activation test by flow cytometry: recent developments in clinical studies, standardization and emerging perspectives. Clin Mol Allergy 2005;30:3-9.
12. Gober LM, Echman JA, Sterba PM, Vasagar K, Schroeder JT, Golden DBK, et al. Expression of activation markers on basophils in a controlled model of anaphylaxis. Allergy Clin Immunol 2007;119:1181-8.
13. Erdmann SM, Heussen N, Moll-Slodowy S, Merk MF, Sachs B. CD63 expression on basophils as a tool for the diagnosis of pollen-associated food allergy: sensitivity and specificity. Clin Exp Allergy 2003;33:607-14.
14. Stirling RG, Chung KF. New immunological approaches and cytokine targets in asthma and allergy. Eur Resp J 2000;16:1158-74.
15. Kawakasis T, Galli SJ. Regulation of mast-cell and basophil function and survival by IgE. Nat Rev Immunol 2002;2:773-86.
16. Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. Curr Opin Immunol 2000;12:624-31.
17. Obata K, Mukai K, Tsujimura Y, Ishiwata K, Kawano Y, Minegishi Y, et al. Basophils are essential initiators of a novel type of chronic allergic inflammation. Blood 2007;110:913-20.
18. Iikura M, Ebisawa M, Yamaguchi M, Tachimoto H, Ohta K, Yamamoto K, et al. Transendothelial migration of human basophils. J Immunol 2004;173:5189-95.
19. Valent P, Besemer Muhm JM, Majdic O, Lechner K, Beltelheim P. Interleukin 3 activates human blood basophils via high-affinity binding sites. Proc Natl Acad Sci USA 1989;86:5542-6.
20. Szegedi A, Irinyi B, Gál M, Hunyadi J, Dankó K, Kiss E, et al. Significant correlation between the CD63 assay and the histamine release assay in chronic urticaria. Br J Dermatol 2006;155:67-75.
21. Casolaro V, Georas SN, Song Z, Ono SJ. Biology and genetics of atopic disease. Curr Opin Immunol 1996;8:796-803.
22. Busse WW. Mechanisms and advances in allergic diseases. J Allergy Clin Immunol 2000;105:593-8.
23. Patella V, Florio G, Petraroli A, Marone G. HIV-1 gp120 induces IL-4 and IL-13 release from human FcεRI+ cells through interaction with the VH3 region of IgE. J Immunol 2000;164:589-95.
24. Becker Y. A point of view: HIV-1/AIDS is an allergy but CpG ODN treatments may inhibit virus replication and reactivate the adaptive immunity--hypothesis and implications. Virus Genes 2005;30:127-31.
25. Becker Y. HIV-1 induced AIDS is an allergy and the allergen is the shed gp120--a review, hypothesis and implications. Virus Genes 2004;28:319-31.
26. Suzukawa M, Hirai K, Likura M, Nagase H, Komiya A, Yoshimura-Uchiyama C, et al. IgE- and FCeRI-mediated migration of human basophils. Int Immunol 2005;17:1249-55.
27. Min B, LeGros G, Paul WE. Basophils: a potential liason between innate and adaptive immunity. Allergol Int 2006;55:99-104.
28. DePaulis A, Florio G, Prevete N, Triggiani M, Fiorentino I, Genovese A, et al. HIV-1 envelope gp41 peptides promote migration of human FCeRI+ cells and inhibit Il-13 synthesis through interaction with formyl peptide receptors. J Immunol 2002;169:4559-67.
29. Florian S, Sonneck K, Czerny M, Hennersdorf F, Hauswirth AW, Buhring HJ, et al. Detection of novel leukocyte differentiation antigens on basophils and mast cells by HLA-DA 8 antibodies. Allergy 2006;61:1054-62.
30. Macfarlane AJ, Kon OM, Smith SJ, Zeibecoglou K, Khan LN, Barata LT, et al. Basophils, eosinophils and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. J Allergy Clin Immunol 2000;105:99-107.
31. European Respiratory Society. The european lung white book. [Online]. 2003 [cited 2010 Feb 01]; Available from: URL:http://www.ersnet.org/ers/show/default.aspx?id_attach=6106
32. Cell Biology and Cytochemistry. [Online]. 2005 [cited 2010 Feb 01]; Available from: URL:http://www.cytochemistry.net/microanatomy/blood/more_basophils.html
33. Marone G, Florio G, Petraroli A, TriggianiM,. DePaulis A. Human mast cells and basophils in HIV-1 infection. Trends Immunol 2001;22:229-32.
34. Jinguan T, Jacobi HH, Jing C, Reimert CM, Quan S, Dissing S, et al. Chemokine stromal cell-derived factor 1a activates basophils by means of CxCR4. J Allergy Clin Immunol 2001;106:313-20.

References continued on page 88

hence prove most beneficial to the patient.

ACKNOWLEDGEMENTS

Funded by Discipline of Physiotherapy, School of Medicine, Trinity College Dublin. The Henry Cooke Drury Student Research Fellowship contributed to this project.
Many thanks to the physiotherapy, clinical nutrition and administrative staff in the centre for Cardiac Rehabilitation, CReST Directorate, St. James's Hospital, Dublin.

APPENDIX

[i] Karvonen Formula: Target Heart Rate = [(max HR – resting HR) × %Intensity] + resting HR
[ii] Using either the IntelliVue TRx 4851A system (Phillips Healthcare, Netherlands) or F1+ Polar monitors (Polar Electro, Finland)
[iii] Upper arm automated BP/HR Monitors (Microlife BP 3VTO-AP, Widnau, Switzerland)
[iv] Seca 222 height measuring instrument (Seca, Germany)
[v] Body Composition Analyser (Tanita BC-420MA, Illinois, USA)
[vi] BMI = [weight (kg) ÷ (height (m))^2]

REFERENCES

1 The Irish Heart Foundation. 50 Years of Heart Disease in Ireland; Morbidity, Mortality and Heath Services Implications, 2001.
2 WHO, Global Strategy on Diet. Physical Activity and Health, 2004.
3 WHO, World Health Report, 2003.
4 Miller TD, Balady GJ, Fletcher GF. Exercise and its Role in the Prevention and Rehabilitation of Cardiovascular Disease. Ann Behav Med. 1997 Summer; 19 (3): 220-9.
5 O'Connor GT, Buring JE, Yusuf S, Goldhaber SZ, Olmstead EM, Paffenbarger RS Jr et al. An Overview of Randomised Trials of Rehabilitation with Exercise after Myocardial Infarction. Circulation. 1989;80(2): 234-244.
6 Alter DA, Oh PI, Chong A. Relationship between cardiac rehabilitation and survival after acute cardiac hospitalization within a universal health care system. Eur J Cardiovasc Prev Rehabil. 2009 Feb;16(1):102-13.
7 Moreira MM, Souza HP, Schwingel PA, Sá CK, Zoppi CC. Effects of aerobic and anaerobic exercise on cardiac risk variables in overweight adults. Arq Bras Cardiol. 2008 Oct;91(4):200-6, 219-26.
8 Ranković G, Milicić B, Savić T, Dindić B, Mancev Z, Pesić G. Effects of physical exercise on inflammatory parameters and risk for repeated acute coronary syndrome in patients with ischemic heart disease. Vojnosanit Pregl. 2009 Jan;66(1):44-8.
9 Agency for Health Care Policy and Research. Cardiac Rehabilitation Guidelines. Silver Spring, Agency for Health Care Policy and Research Publishing Clearinghouse; 1995.
10 Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA, Marcus BH et al. A Statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity) Circulation. 2003;107:3109-3116.
11 McCartney N. Role of resistance training in heart disease. Medicine and Science in Sports and Exercise, 1998 Oct;30(10 Suppl):S396-402.
12 Sparling PB, Cantwell JD, Dolan CM, Niederman RK. Strength training in a cardiac rehabilitation program: a six-month follow-up. Arch Phys Med Rehabil. 1990 Feb;71(2):148-52.
13 Bjarnason-Wehrens B, Mayer-Berger W, Meister ER, Baum K, Hambrecht R, Gielen S; German Federation for Cardiovascular Prevention and Rehabilitation. Recommendations for resistance exercise in cardiac rehabilitation. Recommendations of the German Federation for Cardiovascular Prevention and Rehabilitation. Eur J Cardiovasc Prev Rehabil. 2004 Aug;11(4):352-61.
14 Hansen D, Dendale P, Berger J, Onkelinx S, Reyckers I, Hermans A et al., Importance of exercise training session duration in the rehabilitation of coronary artery disease patients. Eur J Cardiovasc Prev Rehabi 2008 Aug;15(4):453-9.
15 Rivett MJ, Tsakirides C, Pringle A, Carroll S, Ingle L, Dudfield M. Physical activity readiness in patient withdrawals from cardiac rehabilitation. Br J Nurs. 2009 Feb 12-25;18(3):188-91.
16 Thompson DR, Bowman GS. Evidence for the effectiveness of cardiac rehabilitation. Intensive Crit Care Nurs 1998; 14: 38-48.
17 American Association for Cardiovascular and Pulmonary Rehabilitation. Guidelines for Cardiac Rehabilitation and Secondary Prevention Programmes. Champaign, Ill: Human Kinetics Publisher; 1999.
18 National Heart, Lung and Blood Institute. Obesity Education Initiative.
19 Hevey D, Brown A, Cahill A, Newton H, Kierns M, Horgan JH. Four-week Multidisciplinary Cardiac Rehabilitation Produces Similar Improvements in Exercise Capacity and Quality of Life to a 10-week Program, J Cardiopulm Rehabil. 2003 Jan-Feb; 23(1): 17-21.
20 Reid RD, Dafoe WA, Morrin L, Mayhew A, Papadakis S, Beaton L et al. Impact of program duration and contact frequency on efficacy and cost of cardiac rehabilitation: results of a randomized trial. Am Heart J. 2005 May;149(5):862-8.

CONTINUED FROM PAGE 19 - ATOPY & HIV..

35. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol 2006;6:761-71.
36. Kovacs JA, Hiemenz JW, Macher AM, Stover D, Murray HW, Shelhamer J, et al. Pneumocystis carinii pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med 1984;100: 663-71.
37. Vasagar K, Vonakis BM, Gober LM, Viksman A, Gibbons SP, Saini SS. Evidence of in vivo basophil activation in chronic idiopathic urticaria. Clin Exp Allergy 2004;36:770-6.
38. Schleimer RP, MacGlashan DW, Gillespie E, Lichtenstein LM. Inhibition of basophil histamine release by anti-inflammatory steroids. J Immunol 1982;129:1632-6.
39. Vigano A, Principi N, Crupi L, Onorato J, Vincenzo ZG, Salvaggio A. Elevation of IgE in HIV-infected children and its correlation with the progression of disease. J Allergy Clin Immunol 1992;89:68-75.
40. Umetsu DT, DeKruyff RH. Th1 and Th2 CD4+ cells in human allergic diseases. J Allergy Clin Immunol 1997;100:1-6.